

Excitatory and inhibitory effects of tricaine (MS-222) on fictive breathing in isolated bullfrog brain stem

MICHAEL S. HEDRICK AND RACHEL E. WINMILL

Department of Biological Sciences, California State University, Hayward, California 94542

Submitted 15 July 2002; accepted in final form 22 October 2002

Hedrick, Michael S. and Rachel E. Winmill. Excitatory and inhibitory effects of tricaine (MS-222) on fictive breathing in isolated bullfrog brain stem. *Am J Physiol Regul Integr Comp Physiol* 284: R405–R412, 2003. First published October 31, 2002; 10.1152/ajpregu.00418.2002.—This study examined the direct effects of tricaine methanesulfonate (MS-222), a sodium-channel blocking local anesthetic, on respiratory motor output using an in vitro brain stem preparation of adult North American bullfrogs (*Rana catesbeiana*). Bullfrogs were anesthetized with halothane, and the brain stem was removed and superfused with artificial cerebrospinal fluid containing MS-222 at concentrations ranging from 0.1 to 1,000 μM . At the lowest concentration of MS-222, respiratory frequency (f_R) increased significantly ($P < 0.05$), but at higher concentrations, f_R progressively decreased and was abolished in all preparations at 1,000 μM ($P < 0.01$). Respiratory burst amplitude and burst duration were not affected by MS-222. The frequency of nonrespiratory neural activity did not significantly change with the addition of MS-222 below 1,000 μM . These data indicate that MS-222 has a significant, direct effect on respiratory motor output from the central nervous system, producing both excitation and inhibition of fictive breathing. The results are consistent with other studies demonstrating that low concentrations of anesthetics generally cause excitation followed by depression at higher concentrations. Although the mechanisms underlying the excitatory effects of MS-222 in this study are unclear, they may include increased excitatory neurotransmission and/or disinhibition of inputs to the respiratory central pattern generator.

anesthesia; tricaine methanesulfonate; amphibian; central pattern generator; *Rana catesbeiana*

ANESTHESIA is a requirement for any surgical intervention involving vertebrate animals. The most commonly used compound for anesthesia in fishes and amphibians is tricaine methanesulfonate (MS-222), a local anesthetic that is structurally similar to benzocaine, procaine, and lidocaine (19, 29). Local anesthetics, such as benzocaine and MS-222, block action potential generation by altering the gating properties of voltage-gated Na^+ channels (44). Although the efficacy of MS-222 as a general anesthetic for fishes and amphibians is well established (4, 12, 37, 38), the mechanisms and sites of action for MS-222 remain unclear.

Fishes and amphibians exposed to solutions of MS-222 or other local anesthetics at concentrations ranging from 50 to 1,000 mg/l (0.19 to 3.8 mM) undergo respiratory and cardiovascular depression to a surgical plane of anesthesia (1, 12, 13, 16, 33, 34, 40, 43). Owing to its Na^+ channel blocking properties, it is presumed that MS-222 acts within the central nervous system (CNS) to depress respiratory and cardiovascular function, but there is little direct evidence on the effects of MS-222 on the CNS of fishes or amphibians. For example, in the shark *Squalus acanthias*, the degree of anesthesia is not correlated with the concentration of MS-222 within the brain and cerebrospinal fluid (40). There is also evidence that MS-222 inhibits the release of neurotransmitter at the motor end plate of the grass frog *Rana pipiens* (26). These data suggest that MS-222 may be acting as a local, rather than general, anesthetic. In addition, some excitatory actions of MS-222 on ventilation (33) and heart rate (12, 33) have been observed in fish and on heart rate in the toad *Bufo marinus* (38). The excitatory actions of MS-222 on heart rate are hypothesized to result from withdrawal of vagal parasympathetic tone in fish because vagotomy abolishes the tachycardia in response to MS-222 (33). However, in toads, MS-222-induced tachycardia results from activation of spinal cardiac sympathetic efferent activity (38).

The effects of a variety of anesthetics have been used to examine neural activity within the CNS of intact mammals or with brain tissue slices of mammals (2, 9, 20, 24). By contrast, no studies have examined the direct effects of MS-222 on motor activity from the CNS in fishes or amphibians. Thus the primary goal of this study was to examine the direct effects of MS-222 on respiratory motor activity using a brain stem preparation in vitro that generates central respiratory rhythm. Specifically, we wished to test the hypothesis that MS-222 directly affects motoneuronal output from the CNS in a fictively breathing amphibian preparation.

MATERIALS AND METHODS

Animals. North American bullfrogs (*Rana catesbeiana*) of either sex and with a body mass ranging from 308 to 489 g [mean = 396 \pm 52 g (SD), $n = 15$] were used in this study.

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Address for reprint requests and other correspondence: M. S. Hedrick, Dept. of Biological Sciences, California State Univ., Hayward, CA 94542 (E-mail: mhedrick@csu Hayward.edu).

Animals were purchased from a commercial supplier (Charles Sullivan, Nashville, TN) and were kept in fiberglass tanks supplied with fresh tap water at room temperature (20–23°C). All procedures in this study were approved by the California State University Hayward Institutional Animal Care and Use Committee and are consistent with the Institute for Laboratory Animal Research *Guide for the Care and Use of Laboratory Animals* and the American Physiological Society's "Guiding Principles for Research Involving Animals and Human Beings."

In vitro brain stem preparation. For experiments, frogs were anesthetized by placing them in a closed container (1.5 liters) with a cotton gauze that held ~1 ml of the volatile anesthetic 2-bromo-2-chloro-1,1,1-trifluoroethane (halothane; Webster Veterinary Supply, Sterling, MA). Animals lost corneal, toe pinch, and righting reflexes after about 10- to 15-min exposure to halothane. The animal was quickly decerebrated (<5 min) through a hole made in the cranium with a dental drill. The brain stem was exposed, and cold (5°C), oxygenated artificial cerebrospinal fluid (aCSF) was dripped onto the brain stem throughout the entire dissection procedure (15–20 min) to reduce tissue metabolism and maintain tissue viability. The brain stem was removed, placed into a recording chamber (7 ml) ventral side up, and superfused with aCSF of the following composition (in mM): 75 NaCl, 4.5 KCl, 2.5 CaCl₂, 1.0 MgCl₂, 1.0 NaHPO₄, 40 NaHCO₃, and 7.5 glucose (18). The aCSF flowed through the recording chamber at 5–10 ml/min from a gravity-fed reservoir (350 ml) maintained at a temperature of 20–22°C (30). The aCSF reservoir was bubbled with oxygenated, isocapnic (98% O₂-2% CO₂) gas from an electronic mixing flowmeter (Cameron Instrument, model GF-3MP) or from a tank of premixed gas purchased from a commercial supplier.

Respiratory neural output was recorded with glass suction electrodes attached to cranial nerves (CN) V (trigeminal), X (vagus), and XII (hypoglossal). These nerves innervate buccal elevator and depressor muscles in the oropharyngeal region of anurans and are responsible for generating airflow and controlling glottal airflow, associated with small-amplitude, nonventilatory buccal oscillations and larger-amplitude, positive-pressure lung ventilatory events (7, 23). Previous studies with adult amphibian preparations have verified that neural activity from CN V, VII, X, and XII is correlated with breathing movements in intact animals (35). Neural activity was amplified 10,000× (A-M Systems, model 1700), filtered (10 Hz–5 kHz), and moving time averaged (CWE model MA-821-4). Raw and processed signals were simultaneously recorded and stored on a computer (Dell, Pentium 4) that interfaced with a data-acquisition system sampling at 2 kHz (AD Instruments; Powerlab 8/SP).

Experimental protocol. The recording chamber was connected to two identical parallel reservoirs from which the brain stem was superfused with control aCSF or with buffered MS-222 (ethyl 3-aminobenzoate methanesulfonate salt; Sigma catalog A 5040) dissolved in aCSF at different concentrations by switching between the two reservoirs. The pH of the superfusate (aCSF or MS-222 in aCSF) was measured in the reservoir just before superfusion of the brain stem (cf. Ref. 30). Each experiment began with a 45- to 60-min period of equilibration in aCSF before any measurements were taken. The last 10 min of the aCSF superfusion was recorded as the control. After the control recording, the brain stem was superfused with MS-222 dissolved in aCSF. The MS-222 solution was superfused starting with 0.1 μM and increased in 10-fold increments up to 1,000 μM MS-222. Each concentration of MS-222 was superfused for a period of 15 min before switching to the next concentration. The last 10 min of

recorded data was used for analysis. At the end of the MS-222 treatment, the brain stem was returned to control aCSF for up to 3 h during a recovery/washout period.

Data analysis and statistics. Fictive lung breaths were defined by criteria that have been used in other studies (3, 13, 16–18, 34, 43, 45). Respiratory neural bursts were ~1 s in duration and had a ramplike incrementing burst shape. Lung bursts were also distinguished from low-amplitude, high-frequency buccal bursts by their larger amplitude and overall lower frequency. Fictive buccal activity is not always present in the adult bullfrog brain stem preparation and was not included in this analysis. All other neural activity that did not fit the criteria for respiratory bursts was defined as nonrespiratory (cf. Ref. 34) and grouped together for statistical analysis.

For each experiment, measured variables included frequency of respiratory (lung) and nonrespiratory neural bursts and amplitude and duration of respiratory bursts. The data were analyzed with a one-way ANOVA for repeated measures for equal sample sizes (frequency data) or without repeated measures when sample sizes were unequal (amplitude/duration data). When significant differences were detected by ANOVA, a two-tailed Dunnett's multiple comparison test (47) was used for post hoc evaluation of statistical differences between control values compared with data collected during administration of MS-222 and during recovery. Data expressed as a percentage were converted to their arcsine values before statistical analysis (47). The minimal level of statistical significance was taken as $P < 0.05$. Data are given as means ± SE unless otherwise indicated. Statistical and graphical analyses were carried out with commercially available software (GraphPad Prism v. 3.0, San Diego, CA and Igor Pro v. 4.0.1, Wavemetrics, Lake Oswego, OR).

RESULTS

The pH of the control aCSF and MS-222 solutions ranged between 7.75 ± 0.01 and 7.78 ± 0.02 . The pH of the superfusion solutions were not significantly different from each other (ANOVA; $F_{6,99} = 0.4$; $P > 0.8$), indicating that solution pH was not a factor in this experiment.

Figure 1 illustrates the excitatory and inhibitory effects of MS-222 on respiratory motor output in a single experiment. Respiratory frequency increased from ~5 (control) to ~14 bursts/min with the addition of 0.1 μM MS-222. With increasing concentrations of MS-222 there was a progressive reduction in f_R until neural activity was completely abolished at 1,000 μM. After a washout period of ~45 min, respiratory neural activity resumed and f_R was similar to control (Fig. 1, Recovery). The addition of 0.1 μM MS-222 increased f_R in 13 of 15 experiments with increases ranging from 12 to 600% of control (mean 130% of control) followed by a progressive decline of f_R and complete abolition of neural activity at 1,000 μM MS-222 in all experiments.

Figure 2 provides a summary of the dose-dependent effects of MS-222 on fictive breathing. There was a significant effect of MS-222 on f_R (ANOVA; $F_{6,104} = 8.9$; $P < 0.001$). At the lowest concentration of MS-222 (0.1 μM), f_R increased significantly from 4.6 ± 0.9 (control) to 8.5 ± 2.0 bursts/min (Dunnett's test; $q' = 2.72$; $P < 0.05$). Respiratory depression occurred at higher concentrations of MS-222, with burst frequency signifi-

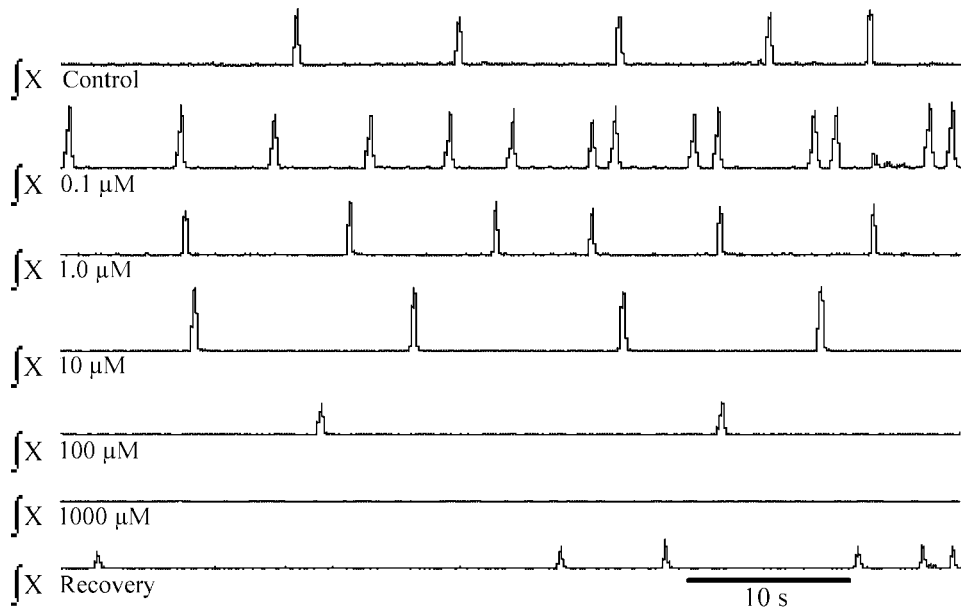


Fig. 1. Effects of MS-222 on integrated vagus nerve (fX) burst activity (fictive breathing) in an isolated brain stem preparation of adult bullfrog. In this experiment, respiratory frequency (f_R) increased with the addition of 0.1 μ M MS-222 and subsequently declined with increasing concentrations of MS-222. All neural activity was abolished at 1,000 μ M and resumed after washout with artificial cerebrospinal fluid (aCSF; Recovery).

cantly attenuated at 100 μ M ($q' = 2.63$; $P < 0.05$) and completely abolished in all 15 preparations at 1,000 μ M ($q' = 3.77$; $P < 0.001$). After exposure to MS-222 solutions, the preparations were returned to control aCSF for 1–3 h for recovery. Most preparations (9 of 15) recovered respiratory-related motor activity in this period after the abolition of breathing at 1,000 μ M MS-222. Respiratory frequency during recovery was slightly lower (2.9 ± 1.0 bursts/min) but not significantly different from control (Fig. 2A; $q' = 1.3$; $P > 0.05$). The effects of MS-222 were limited to changes in frequency, with no significant changes in burst duration (Fig. 2B; ANOVA $F_{5,69} = 0.3$; $P > 0.9$) or burst amplitude (Fig. 2C; ANOVA $F_{5,69} = 1.0$; $P > 0.4$).

During control superfusion with aCSF, 9 of 15 preparations exhibited neural activity that did not fit the criteria established for respiratory (lung) bursts. These were classified as nonrespiratory bursts (see MATERIALS AND METHODS). Examples of nonrespiratory neural activity compared with respiratory bursts in control aCSF and with the addition of 100 μ M are illustrated in Fig. 3. Nonrespiratory burst characteristics were similar to the burst types previously described for the in vitro bullfrog brain stem preparation (34).

The frequency of nonrespiratory bursts was about 0.3–0.6 bursts/min in control, or approximately 1/10th the frequency of respiratory bursts (Fig. 4A). The absolute frequency of nonrespiratory bursts did not change with superfusion of MS-222 below 1,000 μ M (Fig. 4A); however, all neural activity was eliminated at 1,000 μ M MS-222 (Dunnett's test, $q' = 2.26$; $P < 0.05$). Because respiratory bursts progressively declined with increasing MS-222 (Fig. 2A), and nonrespiratory burst frequency did not change, there was a relative increase in the proportion of nonrespiratory bursts with the addition of MS-222 (Fig. 4B; ANOVA $F_{5,73}$; $P < 0.002$). During control superfusion with aCSF, nonrespiratory bursts represented $9.9 \pm 3.6\%$ of

total bursts but increased to $32.2 \pm 10\%$ of total bursts at 10 μ M MS-222 (Dunnett's test, $q' = 3.02$; $P < 0.05$) and represented $44.9 \pm 13\%$ of total bursts at 100 μ M MS-222 (Dunnett's test, $q' = 3.88$; $P < 0.01$). After recovery in aCSF, nonrespiratory bursts were $26.7 \pm 9.4\%$ of total bursts, but this was not significantly different from control (Fig. 4B).

DISCUSSION

The important finding from this study was that MS-222 anesthesia has significant, direct effects on respiratory motor output in the adult bullfrog brain stem in vitro. A low concentration of MS-222 (0.1 μ M) produced a significant increase in fictive breathing in the majority of preparations (13 of 15), with higher concentrations producing attenuation and abolition of respiratory motor output at 1,000 μ M. The effects of MS-222 were limited to changes in respiratory frequency, with no significant changes in other burst characteristics such as burst duration or burst amplitude.

To test the direct effects of MS-222 in this preparation, we initially anesthetized bullfrogs with halothane for removal of the brain stem. Volatile anesthetics are used routinely in clinical medicine with humans and for research with other mammals but have had limited use in amphibians (31). Volatile anesthetics, including halothane, have been used for anesthetization of semi-terrestrial adult *Ranid anurans* (6), aquatic tadpoles (10), and aquatic species such as the African clawed frog, *Xenopus laevis* (39). The advantages of using halothane in this preparation include a wide margin of safety and a short washout time compared with MS-222. In all preparations, respiratory motor activity was present immediately on applying suction electrodes to the cranial motoneurons, illustrating the rapid washout of halothane. In the adult bullfrog brain stem preparation, when MS-222 is used for anesthesia, fic-

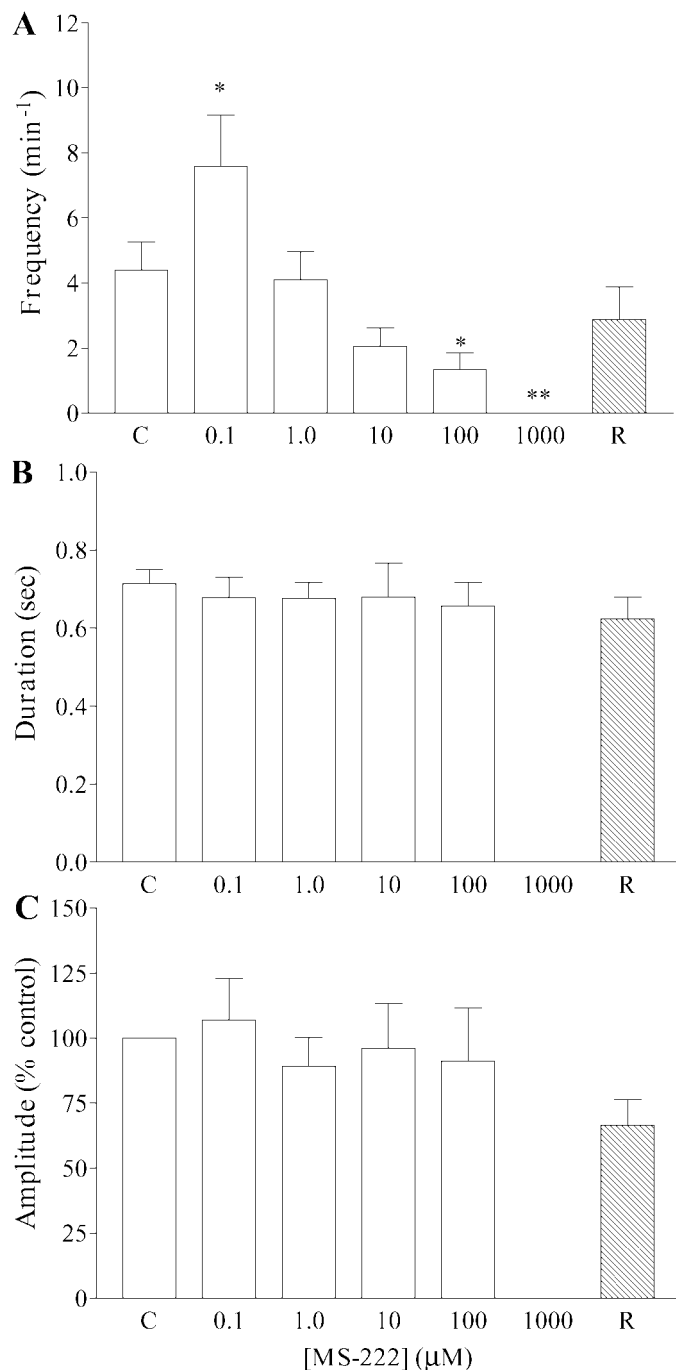


Fig. 2. Respiratory burst frequency is excited and inhibited by MS-222. *A*: there was significant increase in f_R at 0.1 μM MS-222 relative to control, followed by a significant inhibition of f_R at 100 μM and complete abolition of neural activity at 1,000 μM MS-222. *B*: respiratory burst duration was not significantly different in control and increasing concentrations of MS-222. *C*: respiratory burst amplitude, as a percentage of the control amplitude, did not change with increasing MS-222. Values are means \pm SE ($n = 15$). * $P < 0.05$, ** $P < 0.001$ compared with control (2-tailed Dunnett's test). C, control; R, recovery.

tive breathing often does not begin immediately on placing the preparation in the recording chamber (personal observations), which we interpret as the residual effects of inhibition of motor activity with MS-222. In

intact animals, after anesthetization with benzocaine, recovery of spontaneous respiratory activity may require up to 45–60 min (1). Despite these potential drawbacks in the use of MS-222, fictive breathing after halothane anesthesia in the present study (4–5 bursts/min) is not different from several studies where MS-222 was used for anesthetization and removal of the brain stem in adult bullfrogs (3, 17, 18, 21, 34).

Effects of MS-222 on respiratory burst activity. A surprising result from this study was the consistent and significant increase in f_R with the addition of a low concentration of MS-222. This initial increase was followed by consistent attenuation of f_R at concentrations $>0.1 \mu\text{M}$ MS-222. The reduction of fictive breathing with exposure to MS-222 at higher concentrations was not surprising given that intact animals undergo fairly rapid induction to a surgical plane of anesthesia, with loss of breathing movements, righting, and nociceptive reflexes (cf. Ref. 1). Intact animals are typically immersed in MS-222 solutions at concentrations ranging from ~ 30 to 1,500 mg/l (~ 0.1 to 6 mM) for induction of anesthesia (1, 3, 12, 16–18, 29, 34, 38). Although the concentrations used to produce anesthesia in intact animals correspond with concentrations that produce significant depression and abolition of fictive breathing in the isolated brain stem, it is difficult to compare the concentrations used in these different preparations, owing to differences in metabolism, routes of anesthesia, and lack of a blood supply in the brain stem preparation. However, it seems likely that the concentration of MS-222 that produces excitation (0.1 μM) is a subanesthetic dose and is well below the concentrations used for general anesthesia in amphibians.

Although one study (33) has noted excitatory effects of MS-222 on ventilation and heart rate in tench (*Tinca tinca*), the present study is the first to demonstrate direct excitatory and inhibitory effects of MS-222 on respiratory motor output from the CNS. The excitatory effects of MS-222 on ventilation and heart rate occurred at about 100–800 μM MS-222 (33). The excitatory effects on ventilation and heart rate in the tench are thought to be due to inhibition of vagal tone, because bilateral vagotomy abolished the increased respiration and heart rate with MS-222 exposure (33). In toads, spinal, but not brain, pithing or bretylium exposure blocked the tachycardia resulting from MS-222 exposure (38), suggesting that increased sympathetic activation at the level of the spinal cord is responsible for cardiovascular actions of MS-222 in toads.

Excitation of fictive breathing at low doses has implications for animals undergoing and emerging from anesthesia or that are used under lightly anesthetized conditions. For example, fictive breathing in decerebrate, paralyzed, and artificially ventilated toads (*Bufo marinus*) is generally higher in animals that have immediately emerged from MS-222 anesthesia compared with those animals that are allowed to recover from anesthesia for 24–48 h (27). Lightly anesthetized, spontaneously breathing channel catfish (*Ictalurus punctatus*) have respiratory responses to aquatic hyp-

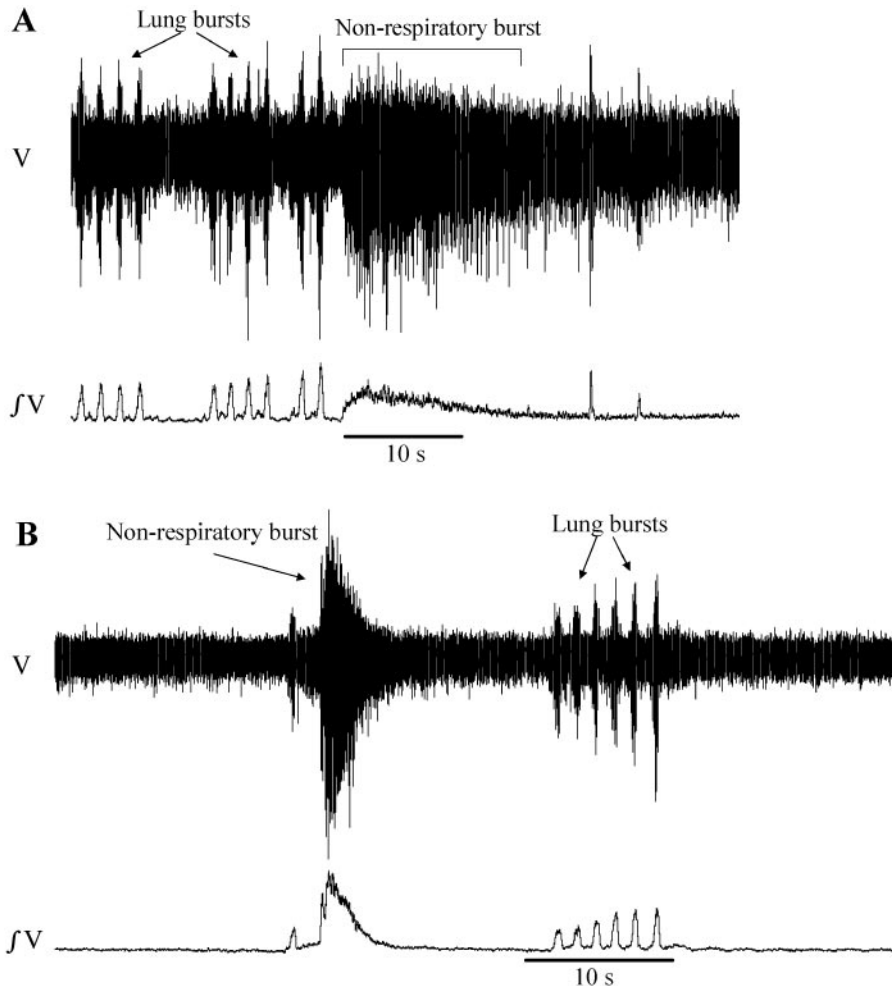


Fig. 3. Respiratory and nonrespiratory neural activity recorded from the trigeminal nerve as raw (V) and integrated (f/fV) activity. Both respiratory and nonrespiratory activity are present during superfusion with control aCSF (A) and 100 μ M MS-222 (B).

oxia or hyperoxia that differ considerably from those of conscious animals (4). Thus MS-222 anesthesia may affect a number of physiological processes, including resting ventilation, heart rate, and peripheral and central reflexes.

In the present study, we cannot distinguish the effects of MS-222 acting at the level of the respiratory central pattern generator or directly on cranial motoneurons. Excitation (and depression) of neural activity occurred simultaneously in all cranial nerves (trigeminal, vagus, and hypoglossal) and was not limited to the vagus nerve, suggesting a direct effect on the respiratory central pattern generator. In addition, the lack of effect of MS-222 on other burst characteristics, such as lung burst duration and burst amplitude (Fig. 2), suggests that MS-222 has a direct effect on the neural networks that affect rhythm generation, rather than those that affect respiratory burst pattern formation (cf. Ref. 28).

Several studies with other types of anesthetics have shown dose-dependent effects that produce excitation at low concentrations and depression at higher concentrations, similar to the effects we obtained with MS-222. For example, pentobarbital sodium enhances stimulus-evoked field potentials in rat hippocampus at

concentrations of 1–5 μ M but depresses field potentials at doses >10 μ M (2). Volatile anesthetics and barbiturates enhance nociception at subanesthetic doses (22). Lidocaine and other local anesthetics can cause seizures (widespread CNS excitation) at low doses and CNS depression at high doses (8). The mechanisms underlying the excitatory effects of anesthetics include dose-dependent enhancement of synaptic transmission (2) and/or release of excitatory neurotransmitters such as glutamate (20), or inhibition of inhibitory neurotransmitters such as GABA (15). Glutamate produces excitation of fictive breathing in the bullfrog brain stem (45), whereas GABA inhibits respiratory motor activity in this preparation (3).

The mechanisms producing the biphasic (excitation/inhibition) effects on fictive breathing in the bullfrog are unclear. MS-222 blocks inward Na^+ currents in the isolated squid axon (11), but the concentrations used in that study (1–3 mM) also abolish fictive breathing in the present study. Benzocaine and tricaine block voltage-gated Na^+ channels in the inactivated state (44), which differs from the inhibitory effects of other local anesthetics, such as lidocaine and cocaine, which block voltage-gated Na^+ channels in the open state (44). Voltage-gated Na^+ channels from different tissues also

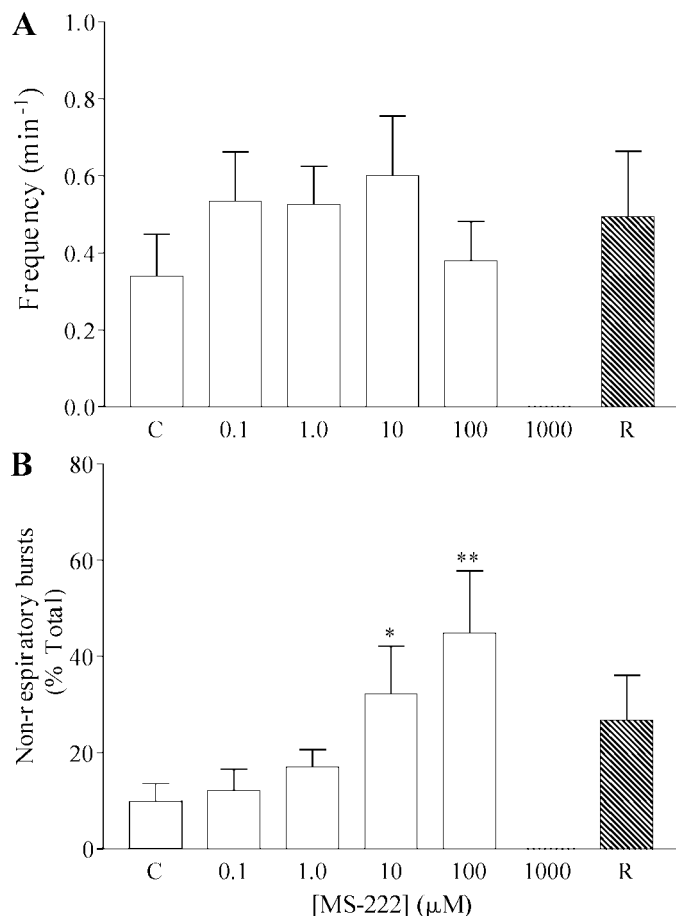


Fig. 4. Summary of nonrespiratory burst activity. *A*: nonrespiratory burst frequency is shown with increasing concentrations of MS-222. *B*: relative proportion of nonrespiratory bursts (%total bursts) is shown. There was a significant relative increase in the percentage of nonrespiratory bursts to total bursts owing to the reduction in respiratory bursts (see Fig. 2*A*). Values are means \pm SE ($n = 15$). * $P < 0.05$, ** $P < 0.01$ relative to control (2-tailed Dunnett's test).

exhibit varying sensitivities to local anesthetics with cardiac Na^+ channels being more sensitive than nerve or skeletal muscle Na^+ channels (25). These observations explain how MS-222 and other local anesthetics would cause respiratory depression, but the excitatory response is more difficult to explain.

A likely cause of the MS-222-induced excitation of fictive breathing in the present study is the interaction of MS-222 with other membrane-bound proteins and/or channels, rather than a direct effect on Na^+ channels. There is substantial evidence that local anesthetics activate a variety of membrane-bound proteins (5); many of these proteins activate second messenger pathways involved with the control of breathing in vertebrates. Other evidence suggests that local anesthetics are capable of blocking K^+ and Ca^{2+} currents and are not limited to blockade of voltage-gated Na^+ channels (5). Local anesthetics are also capable of blocking postsynaptic nicotinic acetylcholine receptors at the neuromuscular junction (5). This would suggest that local anesthetics, including MS-222, might act both centrally and peripherally during anesthetiza-

tion. Peripheral blockade resulting in neuromuscular inhibition might obscure excitatory effects of MS-222 in the CNS. Clearly more studies will have to be done to determine whether the excitatory effects of MS-222 occur at the level of ion channels, synapses, or neural networks associated with breathing in amphibians.

Nonrespiratory burst activity. Several types of neural activity in this preparation were clearly different from typical respiratory burst activity and were classified as nonrespiratory bursts (Fig. 3). Previous studies using adult (34) and larval (13) bullfrog brain stem preparations have previously noted nonrespiratory burst behavior. The nonrespiratory burst types recorded in the present study are similar to those characterized by Reid and Milsom (34). In the present study, nonrespiratory bursts occurred at a frequency of <1 burst/min in control aCSF and in MS-222 solutions (Fig. 4*A*). These data indicate that MS-222 is not likely to contribute to the formation of nonrespiratory bursts in this preparation. Furthermore, nonrespiratory bursts are relatively insensitive to MS-222, indicating that nonrespiratory burst activity is probably less sensitive to Na^+ channel blockade. This indicates that respiratory and nonrespiratory bursts are produced by different mechanisms or may be localized to different areas of the brain stem. For example, respiratory bursts in late-stage tadpoles and adult bullfrogs are generated in the rostral brain stem (43, 45), at the level of cranial nerves IX–X, with other nonrespiratory bursts, such as fictive calling, localized to the pretrigeminal nucleus (36). In control conditions, nonrespiratory bursts represented $\sim 10\%$ of total burst activity, but the relative proportion of nonrespiratory bursts increased significantly to 30–45% of total bursts with the addition of higher concentrations of MS-222, owing to the inhibition of respiratory bursts. Similar proportions of nonrespiratory bursts were reported in another study (34).

It is unclear from the present study what types of motor behaviors, if any, are associated with nonrespiratory bursts. Previous studies using isolated adult lamprey brain stem preparations recorded nonrespiratory neural activity that was characterized as fictive "coughs" or "arousal" breathing (41, 42). The burst characteristics of arousals and coughs are very similar to the nonrespiratory bursts recorded in the present study. Fictive arousals and coughs in lampreys exhibit higher-amplitude, longer-duration neural activity than normal fictive breathing, differ in terms of synaptic activation and brain stem connectivity, and can be elicited by sensory stimulation in intact animals (41, 42). Nonrespiratory bursts in the bullfrog brain stem may, therefore, represent a general arousal burst, and this might be important for generating widespread activity to resuscitate breathing within the CNS during anesthesia or other types of respiratory depression such as tissue hypoxia. Arousal bursts, to be effective, would be expected to be less sensitive to Na^+ channel blockade by MS-222 as we have observed in this study (Fig. 4*A*). However, other types of neural output from the CNS in amphibians, which cannot be discounted,

include neural activity associated with vocalization (36, 46) and feeding (32). Further studies will have to be done to identify the putative motor behaviors associated with the different types of nonrespiratory neural bursts generated by the isolated amphibian brain stem.

In conclusion, MS-222 has direct, dose-dependent effects on the adult bullfrog brain stem preparation, including stimulation and depression of fictive breathing. These effects appear to be limited to changes in respiratory frequency because MS-222 does not appear to affect respiratory burst duration, amplitude, or the frequency of nonrespiratory neural activity. We hypothesize that low (0.1 μM), subanesthetic concentrations of MS-222 stimulate respiratory-related pathways through an unknown mechanism, but higher concentrations of MS-222 block voltage-gated Na^+ channels, resulting in centrally mediated depression of respiratory activity.

We thank Dr. P. E. Bickler (Dept. of Anesthesia, University of California-San Francisco Medical School) for helpful discussions on the use and mechanisms of anesthetics.

This study was supported by National Institutes of Health-Minority Biomedical Research Support/Support for Continuous Research Excellence Grant S06-GM-48135.

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