Selective Suppression of Late Laryngeal Adductor Responses by
N-Methyl-D-Aspartate Receptor Blockade in the Cat

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INTRODUCTION

The laryngeal adductor reflex is a sensorimotor response that prevents the entry of foreign bodies into the trachea. The

internal branch of the superior laryngeal nerve (ISLN) contains the laryngeal mucosal afferent fibers that terminate in the nucleus of the tractus solitarius (NTS) in the cat (Kalina and Mesulam 1980; Nomura and Mizuno 1983; Tanaka et al. 1987) and rat (Mrini and Jean 1995; Patrickson et al. 1991). Laryngeal afferent stimulation results in a robust thyroarytenoid (TA) muscle response producing glottic closure both in cats (Sasaki and Suzuki 1976; Suzuki 1987) and human subjects (Ludlow et al. 1992). Two responses, an early response (R1 at 18 ms) and a late response (R2 at 65 ms) occur with electrical stimulation of the ISLN in awake humans (Ludlow et al. 1992; Yamashita et al. 1997). In the human, R1 is a brief unilateral response while R2 is prolonged and bilateral (Ludlow et al. 1992). The two are distinct; for example, only R1 corresponds to stimulation intensity (Yamashita et al. 1997) and only R2 is suppressed during swallowing (Barkmeier et al. 2000). Furthermore, the early R1 response is not altered by vibratory stimulation while the R2 response is facilitated by the addition of vibratory stimuli to the laryngeal mucosa during ISLN electrical stimulation suggesting central summation effects (Ito 1992).

The laryngeal adductor response is modulated by central mechanisms similar to the blink reflex (Sanes and Ison 1983). When single stimuli are presented in pairs at short intervals of less than 1 s in normal volunteers, the response to the second stimulus of a pair is either reduced in amplitude relative to the first (Ludlow et al. 1995) or does not occur. This demonstrates the possible effect of inhibitory interneurons being invoked by the first stimulus, which then suppress the response to the second stimulus. Postsynaptic responses in the NTS to ISLN stimulation are suppressed in the cat at intervals of less than 500 ms (Sessle 1973b).

Adductor spasmodic dysphonia is a laryngeal dystonia with statistically significant increases in the TA muscles during voice breaks in speech (Nash and Ludlow 1996). Although other muscles may exhibit spasms besides the TA (Bielszowicz and Ludlow 2000; Nash and Ludlow 1996) botulinum toxin injections into this muscle have been effective in controlling symptoms in more than 90% of patients (Blitzer et al. 1998). In an earlier study, we determined whether the uncontrolled muscle spasms might be related to the laryngeal adductor response by studying conditioning effects on the late R2.
response. At interstimulus intervals of 1 s or less, the conditioned responses occurred more often in persons affected with adductor spasmodic dysphonia (Ludlow et al. 1995) and abductor spasmodic dysphonia (Deleyiannis et al. 1999) than in control subjects. If N-methyl-D-aspartate (NMDA) receptor blockade modulates these laryngeal responses, it might have clinical utility in the management of these symptoms.

Immunocytochemical and electrophysiological data suggest that the amino acid glutamate is a prominent excitatory neurotransmitter in afferent pathways of the NTS, the termination zone of laryngeal afferents (Dietrich et al. 1982; Maley 1994; Reis et al. 1981; Rugiero et al. 1994). Our recent immunocytochemical demonstration of both NMDA and non-NMDA receptors in the NTS (Ambalavanar et al. 1998), and previous physiological studies using agonists and antagonists to different glutamate receptor subtypes (Andresen and Yang 1994; Henry and Sessle 1985; Kessler and Jean 1991; Sessle 1973b; Sessle and Henry 1989) demonstrate that glutamate neurotransmission in the NTS involves both NMDA and non-NMDA receptors. In vivo studies demonstrate that NMDA receptors play an important role in modulation of the medullary respiratory network (Haji et al. 1998) as blockade of NMDA receptors impairs inspiratory off-switching causing apneusis in cats (Pierrefiche et al. 1994). This effect was observed even after disconnecting the pneumotaxic center (Haji et al. 1998), suggesting that the NMDA receptors are involved in respiratory reflexes in the brain stem. However, the role of NMDA receptors in laryngeal sensory-motor control is not yet clearly understood.

Ketamine is a fast-acting, noncompetitive blocker of the NMDA subtype of excitatory amino acid receptors (Anis et al. 1983; Bennett et al. 1988; Thomson et al. 1985). Ketamine-induced anesthesia involves the NMDA receptor channel complex, at least to some degree, in mice (Irifune et al. 1992). In humans, subanesthetic levels of ketamine alter sensory perception (Øye et al. 1992). A widely used antidepressive agent dextromethorphan is a noncompetitive NMDA receptor blocker but without anesthetic effects (Church et al. 1989, 1994; Netzer et al. 1993).

Recent studies of NMDA receptor antagonists have indicated depressant effects on polysynaptic flexor reflex pathways without altering motor neuron responses to monosynaptic input (Schwarz et al. 1994). NMDA receptor antagonists have recently received increased attention for their analgesic properties (Fisher et al. 2000; Hewitt 2000) and have been shown to have a role in the suppression of secondary hyperalgesia but not primary hyperalgesia (Ilikjaer et al. 1997). Thus NMDA receptor antagonism seems most effective in polysynaptic central neuronal pathways that mediate sensorimotor responses.

The effect of antitussive agents, including dextromethorphan, on laryngeal responses was examined in cats (Mori and Sakai 1975). Intravenous injections of dextromethorphan did not alter the latency or number of evoked recurrent nerve fiber spikes in response to a single electrical stimulus to the superior laryngeal nerve. The number of afterdischarges in the recurrent laryngeal nerve (RLN) in response to multiple repetitive stimuli to the superior laryngeal nerve (SLN), however, were reduced by 24% with administration of dextromethorphan. Further, RLN fibers normally inhibited by SLN stimulation were not affected by dextromethorphan (Mori and Sakai 1975). The dissociation between the lack of effects on recurrent nerve fiber responses during SLN stimulation and discharges following multiple SLN stimuli may indicate that the shorter R1 pathway may not involve NMDA receptors while the longer R2 pathway may involve NMDA receptor mediation.

We evaluated the effect of systemic administration of ketamine, an anesthetic and a noncompetitive NMDA receptor blocker, on the laryngeal muscle responses, R1 and R2, during electrical stimulation of the ISLN in three studies. We quantified the frequency of occurrence of R1 and R2 responses, the amplitude and latency of these responses, and conditioning effects when paired stimuli were presented at interstimulus intervals of 250 ms. In the first experiment, cats anesthetized with and without ketamine premedication were compared on percent occurrence, latency, and conditioning effects. In the second study, we compared ketamine’s effects on measures of response occurrence, amplitude, and conditioning effects with the effect of increasing the depth of anesthesia. Finally, in the third experiment, dextromethorphan, a nonanesthetic and a noncompetitive NMDA receptor blocker, was used to evaluate the effects of NMDA receptor antagonism on laryngeal sensorimotor response occurrence, amplitude, and conditioning effects without increasing the depth of anesthesia.

**METHODS**

The animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals, prepared by the National Academy of Sciences and published by the National Institutes of Health (National Institutes of Health Publication No. 86-23, revised 1985).

**Experiment 1**

Nine cats, of either sex, were divided into two groups. Four cats were anesthetized initially with a mixture of ketamine (20 mg/kg im) and acepromazine (0.1 mg/kg im, group 1a, n = 4), whereas five cats received only acepromazine (0.1 mg/kg im group 1b, n = 5). Anesthesia was maintained with α-chloralose (40 mg/kg, 10 mg/ml solution iv) in both groups throughout the experiment. A tracheostomy cannula was inserted as low as possible into the trachea to avoid stimulation of the larynx, and spontaneous breathing was maintained with supplemental oxygen (100% O₂, 2 l/min). A femoral artery was cannulated to monitor arterial blood pressure. The heart and respiratory rates were also monitored continuously with a five-lead electrocardiograph (ECG) monitor and an endotracheal CO₂ monitor, respectively. The heart rate, respiratory rate, and oxygen saturation levels were recorded every 15 min. Animals were shaved and a pediatric ground pad fixed to the back (REM Polyhesive, ValleyLab, Boulder, CO). The internal SLNs (ISLNs) were exposed and secured in an insulated bipolar cuff electrode with a 2 mm ID and 2 mm spacing between the contact points, for electrical stimulation. Bipolar hooked wire EMG electrodes (0.002 in diam) with 1-mm bared tips were inserted into the ipsilateral TA muscle to record laryngeal adductor responses. Bipolar hooked wire electrodes were inserted into the diaphragm under direct visualization through an abdominal incision and used to monitor respiration.

The ISLN was electrically stimulated on one side with square-wave 0.2-ms pulses using a Grass S11 stimulator with a S155 stimulus isolation unit and a CCU1A Grass constant current unit. The threshold current level in micramps was determined for eliciting an R1 laryngeal muscle response using a single pulse and then the current was raised to supramaximal levels for R1 responses, usually between 0.6 and 1 mA. A pilot study confirmed that greater suppression of R1 and R2 responses to afferent conditioned stimuli occurred at intervals of
250 ms or less, similar to that reported by Sessle (1973b). Pairs of stimuli were presented with a 250-ms interstimulus interval (ISI) between the two stimuli. At least a full-minute interval occurred between stimulus pairs to reduce any effect that previous stimulation might have on responses to the first member of a stimulus pair. All stimulus pairs were presented in the middle of expiration to control for any changes that might occur in the laryngeal responses due to the respiratory cycle. Ten sets of paired stimuli were presented, 20 stimuli in total. The electromyographic (EMG) signal amplifiers multiplied the signals between 1,000 and 2,000 times to allow visualization of the EMG signals within ±1 V. The muscle signals and the stimulation trigger were digitized on-line from 50 ms before the stimulus onset to 150 ms after the stimulus using triggered data acquisition with CODAS software (Dataq Instruments, Akron, OH) at 5,000 samples per second with anti-aliasing filtering at 2 kHz (Frequency Devices, Haverhill, MA).

Experiment 2

Six cats, including the five cats in the second group of experiment 1 (group 1b) that did not receive ketamine premedication, were assigned to one of two groups. All cats underwent the initial stimulation and recording as described in the preceding text. Next cats were either injected with ketamine (15–20 mg/kg im, group 2a, n = 3) or received a further dose of α-chloralose (40 mg/kg iv, group 2b, n = 3). Initially the respiratory rate fell for 1–2 min after administration but then returned to prebaseline levels. Once the animal was stabilized in respiration and heart rate 15 min later, 10 sets of paired ISLN stimuli were presented and muscle EMG was recorded. Before beginning stimulation, the supramaximal level for eliciting an R1 response was again checked and used for stimulation. In almost all cases, the same stimulation current level was used post medication as was used before medication.

Experiment 3

Five cats underwent the same procedures as in experiment 1. Twenty sets of stimulation pairs were recorded in each animal yielding a total of 40 responses. Next each animal in group 3 received intravenous dextromethorphan at a dosage of 0.03 mg/kg in a 0.03 mg/ml solution (n = 5). Administration was gradual over 15 min while monitoring heart and respiratory rate. Once the animal was stabilized 15 min later, 20 sets of paired ISLN stimuli were presented and the responses recorded. Before beginning stimulation, the supramaximal level for eliciting the greatest R1 response was again checked and was used for stimulation. In almost all cases, the same current level was used postdextromethorphan as was used predextromethorphan.

Data analysis

Nonrectified thyroarytenoid EMG recordings were displayed using MATLAB software (The MathWorks, Natick, MA; Fig. 1). An R1 response was identified if it was greater than baseline, a complex response, not a single motor unit firing, and occurred between 5 and 20 ms. An R2 response was identified if it was greater than baseline, a complex response, not a single motor unit firing, and occurred between 25 and 40 ms. The onset and offset of each R1 and R2 response were marked by one member of the research team and reviewed by a second member. Only those agreed on by both members of the team were included. A 10-ms interval of baseline activity was marked before the stimulus. The signals were then full-wave rectified, and measures were made automatically using software routines in MATLAB to determine the mean level of baseline activity, the onset latency of a response relative to the stimulus, and the total area under the curve of a response in microvolt-milliseconds after subtraction of baseline activity, and the percent of the unconditioned response amplitude was derived by dividing the area under the curve of the conditioned response (following the 2nd stimulus of a pair), by the
area under the curve of the unconditioned response (following the 1st stimulus of a pair) and multiplying by 100.

A SYSTAT program was used to identify when the mean rectified amplitude of a visually marked response was less than the mean prestimulus baseline activity on the same trial. When such instances were identified, which occurred only a couple of times in the entire data set, the trials were automatically coded as “no responses” by the program. The following measures were computed for each of the R1 and R2 responses in a set of 20 paired stimuli: mean latencies for the unconditioned responses, the mean area under the curve for the unconditioned responses, the percent occurrence of unconditioned and conditioned responses, and for each conditioned response in a set, the mean area under the curve for the conditioned response was divided by the mean area for the preceding unconditioned response and multiplied by 100 to derive the percent of the unconditioned response amplitude. Where no response occurred for either the unconditioned or conditioned member of a pair, no percent of the unconditioned response amplitude could be computed. Mean values for a set of 20 in a cat only included those pairs of stimuli where responses occurred.

The effects of treatments were assessed as the change in R1 and R2 measures of: the percent occurrence of the unconditioned responses, the latency of unconditioned responses, the amplitude of unconditioned responses, and the mean percent of the unconditioned response amplitude used as a measure of response conditioning.

**Statistical analyses**

**EXPERIMENT 1.** Mean values were computed for each animal for each of the measures (see preceding text). To compare *group 1a* (ketamine) with *group 1b* (no ketamine), one-way ANOVAs were computed for each of the six measures described in the preceding text that varied within both groups and had values for at least four animals in each group. To correct for multiple ANOVAs, we computed a corrected *P* value for significance by dividing 0.05 by the number of comparisons made for this experiment (*P* = 0.05/3 = 0.0167).

**EXPERIMENT 2.** Mean values were computed for each animal for the pre- and postadministration of either ketamine or alpha-chloralose for the same six variables described above. A two-way ANOVA was computed with the independent factors of group, *group 2a* (ketamine) versus *group 2b* (alpha-chloralose) and the repeated factor (pre vs. post) and the interaction of the repeated factor and the group factor to determine if the pre-post medication effects differed between ketamine and alpha-chloralose. Two-way ANOVAs were computed for each of the measures that varied within both groups and that had pre and post values for at least four animals in each group. A Bonferroni corrected *P* value was determined by dividing 0.05 by the number of group comparisons made for this experiment (*P* = 0.05/4 = 0.0125).

**EXPERIMENT 3.** The percent occurrence and amplitude (area under the curve) of R1 and R2 responses and conditioning effects on R1 were computed for each animal in *group 3* pre- and postadministration of dextromethorphan. A repeated-measures ANOVA was computed for each of the measures that varied within the five animals before and after dextromethorphan and for which pre- and post measures were available on at least four animals. A Bonferroni corrected *P* value was determined by dividing 0.05 by the number of comparisons made for this experiment (*P* = 0.05/2 ≤ 0.025).

**RESULTS**

**Experiment 1**

The percent occurrence of R1 responses was 100% in both groups (preketamine and without ketamine), and the mean latency of R1 responses did not differ between groups (*F* = 1.133, *P* = 0.323). Similarly the conditioning effects of the second stimulus in a pair producing a possible reduction in R1 response amplitude were similar in both groups (*F* = 1.240, *P* = 0.302). The percent occurrence of R2 responses, however, was significantly reduced in the ketamine premedicated group (*group 1a*) relative to the nonketamine group (*group 1b*; *F* = 10.207, *P* = 0.015) using a Bonferroni corrected criterion *P* level of ≤0.0167 (Fig. 2). This reduction in R2 responses was not found in one of the four cats, however. The only difference in this animal was that the surgery took longer and the time of the study was more than 2 h after the ketamine premedication. There were too few R2 responses in *group 1a* to compare the two groups on R2 latency or conditioning effects. In addition, because of differences in electrode location across muscles and different stimulation levels between animals in the two groups, the mean R1 response amplitude could not be compared across groups.

No differences were found between the ketamine premedicated *group 1a* and *group 1b* without ketamine premedication on heart rate (*F* = 0.016, *P* = 0.902). The respiratory rate tended to be lower in *group 1a*, but this was not significant (*F* = 1.103, *P* = 0.324). Therefore the effects of ketamine on the heart and respiratory rates were less systematic than the suppressive effects of ketamine on R2 responses.

**Experiment 2**

No comparisons were made on the percent occurrence of R1, which was 100% both before and after both ketamine and alpha-chloralose administration in *groups 2a* and 2b. Because the number of R2 responses were too few following ketamine, comparisons of mean R2 latencies and mean R2 amplitudes before and after ketamine could not be analyzed statistically (Fig. 3). The R1 responses, however, were present in all animals in both groups in both conditions. The mean latency of R1 responses did not differ pre versus post ketamine or alpha-chloralose administration (*F* = 0.267, *P* = 0.619) or interact with ketamine versus alpha-chloralose (*F* = 0.366, *P* = 0.562). Similarly, the conditioning effects on R1 amplitude did not differ pre versus post (*F* = 0.864, *P* = 0.380) or interact with ketamine versus alpha-chloralose (*F* = 0.098, *P* = 0.762). R1 response amplitudes (mean area under the curve) did not change pre versus post (*F* = 2.114, *P* = 0.184) or interact with ketamine versus alpha-chloralose (*F* = 0.064, *P* = 0.807). The percent occurrence of R2 responses, however, was significantly (*P* ≤ 0.0125) reduced pre versus post (*F* = 17.576, *P* = 0.003) using a Bonferroni corrected criterion *P* level of ≤0.0125 and showed a nonsignificant tendency to be suppressed to a greater degree in *group 2a* following ketamine than in *group 2b* following alpha-chloralose (*F* = 7.547, *P* = 0.025; Fig. 4).

Heart rate did not change following either medication (*F* = 0.512, *P* = 0.492) and no differences were found between ketamine and alpha-chloralose in the effect on heart rate (*F* = 0.0002, *P* = 0.969). Similarly, the respiratory rate did not change pre versus post (*F* = 0.012, *P* = 0.915) and did not interact with ketamine versus alpha-chloralose (*F* = 0.022, *P* = 0.885), although there was a trend for the respiratory rate to decrease after both agents (Fig. 5). Therefore the suppression of R2 responses was greater than changes in heart or respiratory rates with the administration of ketamine.
Experiment 3

Because the mean percent occurrence of R1 responses was 100% in group 3 before and after dextromethorphan, no comparisons were conducted on this measure. No statistical comparisons could be made for the measures of R2 mean percent of the unconditioned response amplitude and the mean R2 response latency because too few R2 responses occurred following dextromethorphan for statistical comparisons. R2 response occurrence was reduced after dextromethorphan ($F_{1,10} = 9.062$, $P = 0.017$) using a Bonferroni corrected criterion $P$ value of $0.025$ (Fig. 6). Two of the animals did not follow the group direction, one increased in both percent occurrence of R2 responses and response amplitude (Fig. 7). Another showed a reduction in the percent of R2 responses from 65 to 40%, but the amplitudes did not change. These two animals were studied earlier than the other animals, and the duration of the period that we were able to maintain the animal in a stable state following dextromethorphan administration was not as long as in subsequent animals. No significant changes ($P < 0.025$) occurred in the mean amplitude (area under the curve) in R1 responses following dextromethorphan ($F = 1.445$, $P = 0.296$).

Changes in heart rate, respiratory rate, CO$_2$ levels, O$_2$ saturation, and diastolic and systolic blood pressure were compared before and after dextromethorphan. Only diastolic blood pressure was changed with a reduction following the administration of dextromethorphan ($F = 12.145$, $P = 0.001$; Fig. 8) from a mean value of 76.9–61.0 mmHg following dextromethorphan. These data, however, include readings that occurred immediately after the administration of dextromethorphan as well as those made after the animal was stabilized and before beginning the ISLN stimulation study.

**DISCUSSION**

We report for the first time that systemic administration of either ketamine or dextromethorphan abolishes the late R2 laryngeal adductor muscle responses in the cat. These results suggest that the elicitation of the R2 response depends on NMDA receptor activation. We contrasted groups of animals with combinations of alpha-chloralose and other medications. In the first study, the preanesthetic administration of ketamine followed by alpha-chloralose was contrasted with preanesthetic administration of acepromazine combined with alpha-chloralose. Similarly, in the second experiment, alpha-chloralose followed by ketamine was contrasted with increased alpha-chloralose without ketamine. These effects might have in-

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**Figure 2**

A. Top: R1 and R2 responses in a recording from an ipsilateral thyroarytenoid muscle, at approximately 10 and approximately 38 ms, respectively, elicited by electrical stimulation of the right superior laryngeal nerve in an anesthetized cat with alpha-chloralose with no ketamine premedication. The height of the vertical bar shows a signal amplitude of 1,000 µV. Bottom: responses in an ipsilateral thyroarytenoid muscle to electrical stimulation of the right superior laryngeal nerve in an anesthetized cat with alpha-chloralose and ketamine premedication. Note the absence of R2 responses with ketamine premedication. In both panels, the top trace is a response to an unconditioned stimulus followed by the next tracing below, which is a response to a conditioned stimulus presented 250 ms later. Each subsequent pair is an unconditioned response followed by a conditioned response. The height of the vertical bar shows a signal amplitude of 500 µV. B: point plots of the percent occurrence of unconditioned R1 and R2 responses in 2 groups of animals, 5 animals that received only acepromazine for premedication and were maintained on alpha-chloralose (no ketamine) and four animals that received ketamine with acepromazine for premedication and were maintained on alpha-chloralose (ketamine). C: box plots of the heart rate and respiratory rate of the 2 groups of animals, 5 animals that received only acepromazine for premedication and were maintained on alpha-chloralose (no ketamine) and 4 animals that received ketamine with acepromazine for premedication and were maintained on alpha-chloralose (ketamine). The box plots show the quartile distribution around the median, the boxes contain the quartiles above and below the median, while the vertical lines show the quartiles on either side. The crosses plot outlier values.
involved an interaction of alpha-chloralose with ketamine; however, the third experiment included acepromazine, alpha-chloralose, and pre- and postdextromethorphan, which did not include ketamine and found a similar suppression of R2 responses. The effects of adding either ketamine or dextromethorphan to alpha-chloralose, therefore had similar effects on the laryngeal responses: a selective suppression of R2 responses. Although we were able to discount the effects of added anesthetic effects by a comparison with increasing levels of alpha-chloralose in group 2b, we cannot eliminate the possibility of similar interaction effects of ketamine with alpha-chloralose and dextromethorphan with alpha-chloralose accounting for the similar effects on R2 rather the result being solely due to NMDA receptor blockade.

FIG. 3. A: an example of responses in an ipsilateral thyroarytenoid muscle, R1 at approximately 10 ms and R2 at approximately 37 ms elicited by electrical stimulation of the right superior laryngeal nerve in an anesthetized cat with alpha-chloralose with no ketamine premedication. Bottom: responses in an ipsilateral thyroarytenoid muscle to electrical stimulation of the right superior laryngeal nerve in the same animal following the administration of added alpha-chloralose. The height of the vertical bar shows a signal amplitude of 500 μV. B: an example of responses in an ipsilateral thyroarytenoid muscle elicited by electrical stimulation of the right superior laryngeal nerve in an anesthetized cat with alpha-chloralose with no ketamine premedication. Bottom: responses in an ipsilateral thyroarytenoid muscle to electrical stimulation of the right superior laryngeal nerve in the same animal following the administration of ketamine. Note the absence of R2 responses after ketamine injection. The height of the vertical bar shows a signal amplitude of 1,000 μV. In all 4 panels, the top trace is a response is to an unconditioned stimulus followed by the next tracing below, which is a response to a conditioned stimulus presented 250 ms later. Each subsequent pair is an unconditioned response followed by a conditioned response.
Exos in cats (Karius et al. 1991). SLN or intercostal reflexes in the medulla besides the R1 component of the laryngeal adductor responses are not dependent on NMDA neurotransmission. Neurophysiological studies in humans suggest that the R2 component of the laryngeal adductor reflex may be responsible for vocal fold adduction (Ludlow et al. 1992). The suppression of reflex glottic closure under ketamine anesthesia has long been recognized clinically as presenting a risk for aspiration (Taylor and Towey 1971; Taylor et al. 1972) may be due to R2 suppression alone. That is, the R2 component may have an important role in glottic closure. Further, during volitional swallowing in humans, the R1 response does not change while the R2 response is suppressed (Barkmeier et al. 2000), suggesting that this long-latency response is modulated by central pattern generators for swallowing in the medulla (Jean 2001). Using systematic increases in ISLN stimulation levels in humans, we demonstrated that the R1 and R2 responses are independently controlled (Yamashita et al. 1997). The differential effects of NMDA receptor blockade on these two responses further demonstrate the independence of these two responses.

At this time, we can only postulate the anatomical location of the NMDA receptors involved in the control of R2 responses. The late R2 onset latency of approximately 36 ms, and the prolonged duration of this response, approximately 20 ms, suggest a polysynaptic pathway. One report described the occurrence of a rare contralateral late thyroarytenoid response to vibration of the laryngeal mucosa (Mochida 1990). These authors reported that this response disappeared after intercollicular brain stem section in the cat. Using Fos immunocytochemistry, we have previously demonstrated that the neurons of the lateral and dorsolateral regions of the periaqueductal gray (PAG) are activated by stimulation of laryngeal afferents in the ISLN (Ambalavanar et al. 1999). Although other brain structures may be involved in the laryngeal reflex, the NTS and PAG are likely to play a role in laryngeal afferent processing. Laryngeal afferent fibers terminate in the NTS (Yoshida et al. 1992). With ISLN stimulation, Fos is induced in the interstitial subnucleus of the NTS, the lateral tegmental field of the reticular formation, and the nucleus ambiguus (NA) (Gestreau et al. 1997; Tanaka et al. 1995, 1996). The R1 response likely involves interneurons in the solitarius-ambiguous pathway (Jean 2001). Our findings suggest that NMDA receptors are not involved, or do not modulate, activity in this short-latency pathway. These findings are in agreement with our previous comparisons of the density of different glutamate receptor subunits in the NTS (Ambalavanar et al. 1998). Greater densities of cells immunoreactive to non-NMDA receptor subunits (GluR1, GluR2/3, GluR4) were found than for NMDA receptor subunits. Only 10% of the stained cells were immunoreactive for NR1 in the interstitial nucleus of the NTS.

The characteristics of the laryngeal R2 responses are similar to those of the blink reflex. Clinical reports have described a selective loss of the R2 component of the blink reflex following brain stem lesions involving the lateral tegmental field (Aramideh et al. 1997). Recent Fos studies of neuronal activation with ISLN stimulation have reported increased Fos expression in the lateral tegmental field (Tanaka et al. 1996). The same study suggests that interneurons in the lateral tegmental field are also possibly involved in the R2 component of the laryngeal adductor response.

Ketamine is considered a dissociative anesthetic because it suppresses certain higher association and pain pathways with little effect on the lower medullary centers (Anis et al. 1983; Mori et al. 1971; Øye et al. 1992; Sparks et al. 1973, 1975). The inhibition of pain perception by ketamine is due to NMDA channel blockade (Irifune et al. 1992; Øye et al. 1992). The suppression of the laryngeal R2 in cats with ketamine premedication and the reduction in R2 frequency after ketamine injection in this study indicate the involvement of NMDA recep-

FIG. 4. Point plots present the percent occurrence of unconditioned R1 and R2 responses in 2 groups of animals both before and after administration of either alpha-chloralose (left) or ketamine (right). The 3 animals that received only acepromazine for premedication and were maintained on alpha-chloralose (before) and then were administered added alpha-chloralose (after) are shown in the left side graphs. The 3 animals that received only acepromazine for premedication and were maintained on alpha-chloralose (before) and then were administered ketamine (after) are shown on the right side. Top: the frequency of occurrence of R1 responses; bottom: the frequency of occurrence of R2 responses.

The lack of any apparent effect of ketamine or dextromethorphan on the early R1 response is striking and raises the possibility that the R1 response pathway depends primarily on the early R1 response is striking and raises the possibility that the R1 response pathway depends primarily on the early R1 response is striking and raises the possibility that the R1 response pathway depends primarily on the early R1 response is striking and raises the possibility that the R1 response pathway depends primarily on the early R1 response is striking and raises the possibility that the R1 response pathway depends primarily on the early R1 response is striking and raises the possibility that the R1 response pathway depends primarily on the early R1 response is striking and raises the possibility that the R1 response pathway depends primarily on the early R1 response is striking and raises the possibility that the R1 response pathway depends primarily on the early R1 response is striking and raises the possibility that the R1 response pathway depends primarily on the early R1 response is striking and raises the possibility that the R1 response pathway depends primarily on the early R1 response is striking and raises the possibility that the R1 response pathway depends primarily on the early R1 response is striking and raises the possibility that the R1 response pathway depends primarily on the early R1 response is striking and raises the possibility that the R1 response pathway depends primarily on the early R1 response is striking and raises the possibility that the R1 response pathway depends primarily on the early R1 response is striking and raises the possibility that the R1 response pathway depends primarily on the early R1 response is striking and raises the possibility that the R1 response pathway depends primarily on the early R1 response is striking and raises the possibility that the R1 response pathway depends primarily on the early R1 response is striking and raises the possibility that the R1 response pathway depends primarily on the early R1 response is striking and raises the possibility that the R1 response pathway depends primarily on the early R1 response is striking and raises the possibility that the R1 response pathway depends primarily on the early R1 response is striking and raises the possibility that the R1 response pathway depends primarily on the early R1 response is striking and raises the possibility that the R1 response pathway depends primarily on the early R1 response is striking and raises the possibility that the R1 response pathway depends primarily on the early R1 response is striking and raises the possibility that the R1 response pathway depends primarily on the early R1 response is striking and raises the possibility that the R1 response pathway depends primarily on the early
tors in the R2 pathway. Further, the suppression of the R2 response with ketamine was not due to a concomitant increase in the depth of anesthesia because the administration of added alpha-chloralose to increase the depth of anesthesia did not affect the frequency of laryngeal R2 adductor responses. This was further supported by the loss of R2 with the injection of dextromethorphan, a nonanesthetic NMDA blocker (Kamei et al. 1986).

FIG. 5. Box plots of the heart rate (top) and respiratory rate (bottom) of 2 groups of animals. Left: 3 animals that received only acepromazine for premedication and were maintained on alpha-chloralose (before) and then received added alpha-chloralose (after). Right: the animals that received only acepromazine for premedication and were maintained on alpha-chloralose (before) and then received ketamine (after).

FIG. 6. An example of an ipsilateral thyroarytenoid muscle responses, R1 at approximately 10 ms and R2 responses at approximately 35 ms elicited by electrical stimulation of the right superior laryngeal nerve in an anesthetized cat with alpha-chloralose with no ketamine premedication. Bottom: ipsilateral thyroarytenoid muscles to electrical stimulation of the right superior laryngeal nerve in the same animal following the administration of dextromethorphan. Note the absence of R2 responses after dextromethorphan. The height of the vertical bar shows a signal amplitude of 500 µV.
Inhibitory modulations of the glottic closure reflex have been studied using the conditioning paradigm in cats (Sessle 1973a,b) and in humans (Deleyiannis et al. 1999; Ludlow et al. 1995). When paired stimuli are presented to the ISLN in rapid succession, the first stimulus invokes both excitatory mechanisms responsible for the laryngeal adductor response as well as inhibitory interneurons, which then suppress R2 responses to the second stimulus. The conditioning effects were most evident in the R2 responses in the alpha-chloralose-treated group (Figs. 1 and 2A). With alpha-chloralose in the absence of ketamine, the R2 percent occurrence went from 95.8% on the unconditioned trials to 60% on the conditioned trials. The area under the curve of the conditioned R2 responses also decreased to 38.7% of the unconditioned responses with alpha-chloralose in the absence of ketamine. In contrast, R1 responses occurred on 100% of the conditioned and unconditioned responses and no significant decrease in mean area under the curve was found; that is, the conditioned R1 responses were 98% of the unconditioned responses.

Because of the suppression of the unconditioned R2 responses by either ketamine or dextromethorphan, we could not examine whether there was a change in conditioning effects on R2 responses. On the other hand, the conditioning effects on the R1 responses were negligible in the alpha-chloralose conditions and showed no systematic change following ketamine or dextromethorphan injection. This suggests that the effects of NMDA receptor blockade were selective to the mechanisms for the elicitation of late R2 laryngeal adductor responses and did not modify the suppression of conditioned R1 responses or the amplitude, latency, or occurrence of R1 responses. Our results also suggest that the effect of NMDA receptor blockade may be operative postsynaptically beyond the NTS because the effects were selective to R2 responses. Further, because R1 conditioning effects were not altered, ketamine and dextromethorphan are unlikely to have affected inhibitory interneur-
rons in the laryngeal adductor system, similar to previous findings in the spinal mechanisms (Anis et al. 1983).

We found no consistent effects on heart or respiratory rate although there was a slight tendency for a slowing of the respiratory rate due to a prolonged inspiratory phase. This was expected given that the apneustic effect of NMDA receptor blockade only occurs when there is a loss of vagal pulmonary afferent feedback (Czyzok-Krzeska and Lawson 1991; Feldman et al. 1992; Foutz et al. 1989; Pierrefiche et al. 1992, 1994). The lack of significant change in respiratory rate could be expected because vagal afferent feedback was intact in our animals.

Finally, our results may suggest possible clinical application. Patients with spasmodic dysphonia, one type of laryngeal dystonia, suffer from uncontrolled bursts of the thyroarytenoid muscle. These spasmodic muscle bursts interfere with vocal fold vibration producing voice breaks (Nash and Ludlow 1996). Studies of R2 responses in awake humans have shown that conditioned R2 responses are normally suppressed when the second stimulus follows the first within 2 s (Ludlow et al. 1995). In patients with adductor spasmodic dysphonia, however, suppression of R2 responses is decreased (Ludlow et al. 1995). This reduced suppression may indicate that muscle bursts in these patients are the result of hyper-reactive R2 responses to sensory stimulation during voicing. Dextromethorphan is already well recognized as an antitussive agent (Church et al. 1989). The results of this investigation suggest that it might also be useful in the management of laryngeal motor control disorders such as adductor spasmodic dysphonia.

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