Influence of Baclofen on Laryngeal and Spinal Motor Drive During Cough in the Anesthetized Cat

Daniel Castillo, BS; Teresa Pitts, PhD

Objectives/Hypothesis: The antitussive properties of (±) baclofen on laryngeal muscle activities have not been determined. The hypothesis of this study was that administration of (±) baclofen would suppress upper airway muscle motor activity in a dose-dependent manner during cough.

Study Design: This is a prospective, preclinical, hypothesis-driven, paired design.

Methods: Electromyograms of the parasternal, rectus abdominis, thyroarytenoid, posterior cricoarytenoid, and thyrohyoid were measured, along with esophageal pressure. Cough was elicited by mechanical stimulation of the lumen of the intrathoracic trachea in spontaneously breathing cats.

Results: Baclofen (±) (3–10 μg kg⁻¹ i.a.) induced decreases in the electromyogram amplitude of the rectus abdominis motor drive during coughing, the inspiratory and active expiratory (E1) phases of cough, and cough number per epoch. There was no effect of (±) baclofen on the EMG amplitudes of any of the laryngeal muscles, the parasternal, or the duration of the passive expiratory (E2) phase.

Conclusions: Results from the present study indicate differential control mechanisms for laryngeal and inspiratory motor drive during cough, providing evidence of a control system regulating laryngeal activity and inspiratory spinal drive that is divergent from the control of expiratory spinal motoneurons.

Key Words: Cough, baclofen, thyrohyoid, parasternal, rectus abdominis, thyroarytenoid, posterior cricoarytenoid, antitussive, upper airway, larynx.

Level of Evidence: N/A.

INTRODUCTION

Cough is a coordinated protective airway reflex that generates vigorous and rapid airflow, clearing the airway of foreign bodies from the respiratory tract.1–6 While the cough reflex is usually beneficial, there are scenarios where overexpression of this behavior induces significant morbidity. Chronic cough can result from asthma, upper airway viral infection, chronic bronchitis and other chronic respiratory diseases, and/or gastroesophageal reflux disease.7–8 Regardless of origin, chronic cough may result in unpleasant irritation in the throat or chest, which can be painful; chest tightness; hoarse voice; dysphonia and other vocal cord dysfunction symptoms; and acid reflux symptoms.7 In more extreme cases, patients with vigorous chronic cough can develop hiatal or inguinal hernias or fractured ribs.8 Other physical symptoms such as syncope, urinary incontinence, emesis, headaches, and exhaustion can also be caused by chronic cough.7,8 These effects often result in social interference, embarrassment, and withdrawal.9

Antitussive drugs are often prescribed for acute and/or chronic disorders in which cough persists after the underlying condition is resolved. However, clinically available antitussives have not been effective in treating chronic cough.10–20 Centrally acting cough suppressants including opioid and opioid derivatives are considered to be the most effective antitussives; however, there are mixed results with even the most commonly prescribed antitussive codeine and the analgesic/sedative-free dextromethorphan.10–20 Opioids are also associated with side-effects such as physical dependence and respiratory depression.17,21

Baclofen, a centrally acting GABA-Breceptor agonist, has been traditionally used as a treatment for muscle spasticity in patients with spinal cord injuries or degenerative disorders such as multiple sclerosis.22–27 More recently, baclofen has been used to treat cerebral palsy spasticity.27–32 Belvisi et al. (1989) showed that GABA has an effect on neutrally evoked bronchoconstriction in the anesthetized guinea pig via GABA-B receptors.33 Bronchoconstriction is a consistent secondary component and an indication of cough as it increases airflow velocity.4,34 Baclofen has antitussive properties in cat and guinea pig models.35–38 Bolser et al. (1999) found that baclofen decreases primary expiratory motor drive and cough number in a dose-dependent manner without significantly altering primary inspiratory motor drive or total cough cycle time in cats.39 However, the effect of baclofen on laryngeal motor drive, particularly the intrinsic and extrinsic laryngeal muscles, has not
been studied. The purpose of this study was to determine the effect of the centrally active antitussive drug baclofen on the cough motor drive and patterns.

**MATERIALS AND METHODS**

Experiments were performed on five spontaneously breathing male adult cats (mean body weight 4.88 ± 0.71 kg). The animals were anesthetized with sodium pentobarbital by an initial intravenous dose of 35–40 mg kg⁻¹ with supplementary doses of 1–3 mg kg⁻¹ i.v. given as needed. Atropine sulfate (0.1 mg kg⁻¹, i.v.) was administered to reduce airway secretions. The animals were tracheotomized and the right femoral artery and vein were cannulated to monitor arterial blood pressure. End-tidal CO₂, body temperature, and blood gas composition were continually monitored and maintained at physiologic levels. Bipolar insulated fine-wire electrodes were inserted into the parasternal, rectus abdominis, posterior cricoarytenoid, thyroarytenoid, and the thyrohyoid muscles to record electromyographic (EMG) activity. Because the anterolateral abdominal muscles are activated together as a unit during cough,⁴⁰ only the rectus abdominis was recorded in this study. An esophageal balloon was placed to record esophageal pressure (mm Hg). Correct electrode position was confirmed by visual inspection and EMG activity patterns during breathing and cough. A cough was defined as a parasternal EMG bursting with negative inflection in esophageal pressure, followed by rectus abdominis EMG bursting with a positive inflection in esophageal pressure.

**Conditions**

There were three conditions for all animals: the first condition was the control condition with no administered (±) baclofen; condition 1 was (±) baclofen 3 μg kg⁻¹ i.a.; and condition 2 was (±) baclofen 10 μg kg⁻¹ i.a. Condition 2 was a cumulative dose with an additional 7 μg kg⁻¹ i.a. to the 3 μg kg⁻¹ i.a. given during condition 1. Baclofen (±) doses were separated by approximately 10 minutes to allow for distribution of the drug throughout all body compartments and completion of all cough trials.

**Cough Stimulus and Phases**

To evoke tracheobronchial cough, the extra- and intrathoracic trachea was mechanically probed with a length of polyethylene tubing repetitively over the stimulus period. Note that due to the tracheostomy there was no laryngeal stimulation during the cough trials. The stimulation consisted of three trials of 20 seconds of mechanical stimulation, with a 1 minute-interstimulus interval. Before the recorded condition trials, approximately 20 consecutive cough trials were conducted to establish a stable baseline response. We used the cough phase duration defined in Wang et al.⁴¹ (Fig. 1). The inspiratory phase (CT₁), the expiratory phase with active muscle activity (CT₂), the passive expiratory phase (CT₃), and the total cough (CTtot) durations were measured. Duration measures are expressed in milliseconds (ms). CT₁ was defined as the onset of parasternal activity to the maximum burst of the parasternal EMG; CT₂ was defined as the maximum burst of the parasternal EMG to the end of the rectus abdominis EMG activity; and CT₃ was defined as the end of the rectus abdominis activity to the onset of the parasternal EMG activity for the next cough in the epoch.

**Data Processing and Statistical Analysis**

“Spike 2” Version 7 (Cambridge Electronic Design, United Kingdom) was used to automate the analysis process. For duration measures, a mean of the peak noise for 1 second preceding the first cough in the series was set as a threshold marker. The duration measures were marked as onset when the signal was greater than the threshold level and as completion when the signal was again less than or equal to the threshold level. The amplitude measures were marked as the largest amplitude during the EMG burst duration. To enable comparison across animals, EMG data were normalized (% of maximum) in each experiment to the maximum burst during coughing. Differences in dependent variables were analyzed by repeated measures ANOVA with Dunnet’s post hoc test. Data are expressed as mean ± standard error with P < 0.05 considered significant.

Baclofen (±) and atropine sulfate were obtained from Sigma-Aldrich (St. Louis, MO) and pentobarbital sodium was obtained from Lundbeck, Inc. (Deerfield, IL). Doses were calculated as their free base.

**RESULTS**

We conducted 45 cough trials in five animals. Administration of (±) baclofen (3 and 10 μg kg⁻¹, i.a.) resulted in a decrease in the number of coughs and the magnitude of abdominal motor bursts and cough-related esophageal pressures.

**Description of Cough Response**

Coughing was represented by large ramp-like inspiratory bursts, immediately followed by bursting in the rectus abdominis muscle EMG that was ballistic-like (rate of rise equivalent to rate of decrement). Figure 1 shows the activity of the parasternal, rectus abdominis, thyroarytenoid, and thyrohyoid muscle EMGs during tracheobronchial cough. The thyroarytenoid muscle EMG bursts across the cough inspiratory-expulsive phase transition were consistent with its role in laryngeal adduction for

![Figure 1. Example of thyrohyoid activity during a single cough. EMG signals were recorded for the parasternal, rectus abdominis, posterior cricoarytenoid, thyroarytenoid, and thyrohyoid muscles. The inspiratory phase is from the onset of the inspiratory activity to its peak activity. The active expiratory phase (E1) is from the peak of the inspiratory activity to the end of the rectus abdominis activity. The expiratory phase (E2) is from the end of the active expiratory activity to the onset of the next inspiratory burst.](image-url)
the compression phase (Fig. 1). The thyrohyoid EMG activity pattern was coincident with the thyroarytenoid (laryngeal adductor), and also had a ballistic-like burst. In one animal, thyrohyoid activity began later during the active expiratory phase (E1) than the adductor discharge.

Figure 2 is an example of the effects of increasing doses of intraarterial (±) baclofen on esophageal pressure and respiratory muscle EMG magnitudes. The control record shows respiratory muscle EMG and esophageal pressure responses to repetitive coughing in response to mechanical stimulation of the extra- and intrathoracic trachea. When cough did occur after (±) baclofen administration, the magnitudes of parasternal and posterior cricoarytenoid were relatively unchanged. (Fig. 2). However, thyroarytenoid and thyrohyoid EMG magnitudes appeared to be reduced, and this trend was not statistically significant (Table I).

**Effect of Baclofen on Cough Amplitudes**

Table I summarizes the magnitudes of the EMG moving averages during the control period and the two doses of (±) baclofen. Baclofen (±) had a significant dose-dependent effect on rectus abdominis (RA) EMG amplitude during cough \( (P < 0.02) \). Post-hoc tests revealed differences between control \( (53 \pm 6) \) and 3 \( \mu g/kg \) (±) baclofen \( (30 \pm 7, P < 0.05) \) and 10 \( \mu g/kg \) (±) baclofen \( (18 \pm 3, P < 0.01) \). There was no effect on the percent maximum EMG amplitude of the parasternal \( (P = 0.17) \), posterior cricoarytenoid \( (P = 0.11) \), the thyroarytenoid \( (P = 0.39) \), or the thyrohyoid \( (P = 0.62) \) during cough.

**Effect of Baclofen on Cough Phase Durations**

Baclofen (±) administration also significantly prolonged the duration of CT1 \( (P < 0.05) \). Post hoc tests revealed significant differences between control \( (785 \pm 83) \) ms and 10 \( \mu g/kg \) (±) baclofen \( (1169 \pm 115) \) ms, \( P < 0.05) \). CT_E1 was also affected \( (P < 0.02) \), and with control \( (377 \pm 60) \), \( P < 0.01) \) significantly different from the 10 \( \mu g/kg \) (±) baclofen \( (117 \pm 25) \) ms, \( P < 0.01) \). CT_E2 \( (P = 0.25) \) and CT_TOT \( (P = 0.28) \) did not exhibit a similar effect. Cough number per epoch \( (P < 0.02) \) was also affected, and there was a significant difference in cough number between the control condition \( (10.3 \pm 1.2) \) and 10 \( \mu g/kg \) (±) baclofen \( (3.6 \pm 1.495, P < 0.01) \).

**DISCUSSION**

This is the first report a) that the extrinsic laryngeal muscle, the thyrohyoid, is active during coughing and b) of the effects of (±) baclofen on the activity of laryngeal muscles (thyrohyoid, posterior cricoarytenoid, and the thyroarytenoid) during this behavior. Baclofen (±) significantly reduced cough number and abdominal expiratory muscle EMG magnitudes, but it had no effect on the amplitudes of laryngeal muscles during coughing. Lastly, (±) baclofen affected specific cough phase durations, lengthening the inspiratory phase and shortening the active expiratory phase (E1), but did not significantly change the total cough phase duration.
The thyrohyoid is an extrinsic laryngeal muscle (innervated by a branch of cervical nerve one; C1) that upon contraction elevates the larynx, which can be used to stiffen the laryngeal complex during dynamic respiratory behaviors. This muscle was activated in ballistic-like fashion during cough, coincident with the thyroarytenoid (an expiratory phasic laryngeal adductor). We propose that with stronger expiratory drives during coughs, the thyrohyoid acts to proportionally stabilize the larynx to reduce turbulence of the upper airway. Higher turbulence during coughing promotes deposition of particles from the lower airway during cough and adherence to the laryngeal/pharyngeal mucosa.

Baclofen \((\pm)\) is a specific GABA-B receptor agonist with a central action on the cough reflex in the cat, suggesting that our results were due to effects of this drug on the brainstem cough control circuit. While acknowledging that GABA-B agonists can inhibit activity of peripheral sensory afferents, the work of Bolser et al.\(^6\)–\(^{51}\) established that \((\pm)\) baclofen given through intraarterial administration at this dosage range has solely central effects. Baclofen \((\pm)\) administration had no significant effect on laryngeal muscle motor drive during cough, although it decreased the magnitudes of abdominal EMG and esophageal pressure. However, recent laryngeal nerve discharge evoked by electrical stimulation of the superior laryngeal nerve (SLN), can be decreased or abolished by administration of antitussive drugs (codeine, dextromethorphan, oxymetazoline, and pentobarbital)\(^{53}\) and NMDA receptor blockade.\(^{54}\) It is probable that the afferent pathways exciting laryngeal motor neurons during SLN stimulation are independent of the cough pattern generator, and that the excitability of these motor neurons during cough is from novel connections within the medullary circuit. Results from the present study indicate differential control mechanisms for laryngeal muscles controlled by the recurrent laryngeal nerve (posterior cricoarytenoid and thyroarytenoid), C1 (thyrohyoid), and inspiratory spinal motor drive versus expiratory spinal motor drive during cough, providing evidence of a control system regulating upper airway activity that is divergent from that controlling drive to thoracic spinal motoneurons.

This study further confirms the work of Bolser et al.\(^{46–49,51}\) demonstrating that 1) \((\pm)\) baclofen decreased abdominal activity while having little effect on parasternal activity during cough, and 2) there was a significant decrease in cough number. It also extended his work by demonstrating that \((\pm)\) baclofen did not significantly change total cough time, but did increase inspiratory phase duration and decreased the duration of the active expiratory phase (E1). Wang et al.\(^{41}\) expanded the cough phase definition to examine not only total cough cycle time, but the CTI, CTE\(_1\), and CTE\(_2\). This work also revealed that the total cough cycle time has a strong linear relationship with CTE\(_2\) and appears to be unrelated to CTI and CTE\(_1\) durations. This may explain why we found no significant change in CTE\(_2\) and total cough cycle time, and confirms Bolser et al.\(^{58}\) finding that there was no significant change in total cough cycle time after administration of \((\pm)\) baclofen. Bolser et al.\(^{55}\) hypothesized the presence of a gating mechanism that controls the excitability of cough motor pattern. These results also provide evidence to suggest that the control of the amplitude of the muscle activation is separately controlled from its duration. Microinjection of baclofen into the NTS in rabbits did increase the cough phase duration without affecting inspiratory amplitude; and Mutolo et al. hypothesized that this was related to a reduced rate of rise of the inspiratory muscles, and thus a inspiratory depressant effect.\(^{56}\)

The caudal nucleus tractus solitarius (NTS) has been hypothesized as a possible action site for the cough gating mechanism.\(^{59,60}\) Furthermore, this gating mechanism may be separated into second-order neuron circuits that control different portions of the cough pattern, for example inspiratory, expiratory, and the compression phase through laryngeal motor control. McGovern et al.\(^{57}\) has shown, through trans-synaptic anatomical tracing in the rat, an average of 3,000 NTS neurons labeled following an injection of herpes simplex virus 1 into the extrathoracic trachea. The transsynaptic labeling progression began in the caudal portion of the NTS, then spread to the medial and dorsolateral subnuclei. The large number of labeled neurons \((\approx 3,000)\) at the 96-hour time point supports a complex processing network of afferent information in the NTS. This putative complex multisynaptic network differs from current hypotheses of a pauci-synaptic network of second-order interneurons in the NTS.\(^{58–61}\)

It is also plausible that many neural structures may respond to i.a. administration of \((\pm)\) baclofen. These effects may be due to low doses of the drug at many brainstem locations, while a similar effect might be obtained by administration of a higher dose within a small area. The drug effect is highly dependent on the route of administration, and the local concentration within a number of brainstem locations following i.a. administration of antitussive drugs has yet to be verified.

**CONCLUSION**

Dicepingitasis\(^{62–64}\) demonstrated the cough suppressive effects of baclofen (decreasing the number of coughs per trial and increasing the cough-response threshold) in healthy subjects, those with cervical spinal cord injury, and those with refractory (i.e., chronic) cough. However, the present study indicates differential control mechanisms for laryngeal muscles, inspiratory muscles, versus expiratory muscle motor drive during cough, providing evidence of a control system regulating upper airway activity which is divergent from that controlling drive to thoracic spinal motoneurons. Future work is needed to further understand the mechanisms that control excitability to the inspiratory muscles, laryngeal muscles, and expiratory muscles in animal and human models. This might elucidate mechanisms for the differential effects of cough suppressants, such as codeine during clinical trials versus preclinical studies. The outcomes of these studies should provide further information about brainstem locations and role of neurotransmitters for the central control of cough.
ACKNOWLEDGEMENTS
We thank Donald Bolser for his help with article preparation, and Melanie Rose for her expert technical assistance.

BIBLIOGRAPHY

Laryngoscope 123: December 2013
Castillo and Pitts: Cough and Baclofen