



Intra-Arterial, but Not Intrathecal, Baclofen and Codeine Attenuates Cough in the Cat

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Centrally-acting antitussive drugs are thought to act solely in the brainstem. However, the role of the spinal cord in the mechanism of action of these drugs is unknown. The purpose of this study was to determine if antitussive drugs act in the spinal cord to reduce the magnitude of tracheobronchial (TB) cough-related expiratory activity. Experiments were conducted in anesthetized, spontaneously breathing cats ($n = 22$). Electromyograms (EMG) were recorded from the parasternal (PS) and transversus abdominis (TA) or rectus abdominis muscles. Mechanical stimulation of the trachea or larynx was used to elicit TB cough. Baclofen (10 and 100 $\mu\text{g}/\text{kg}$, GABA-B receptor agonist) or codeine (30 $\mu\text{g}/\text{kg}$, opioid receptor agonist) was administered into the intrathecal (i.t.) space and also into brainstem circulation *via* the vertebral artery. Cumulative doses of i.t. baclofen or codeine had no effect on PS, abdominal muscle EMGs or cough number during the TB cough. Subsequent intra-arterial (i.a.) administration of baclofen or codeine significantly reduced magnitude of abdominal and PS muscles during TB cough. Furthermore, TB cough number was significantly suppressed by i.a. baclofen. The influence of these drugs on other behaviors that activate abdominal motor pathways was also assessed. The abdominal EMG response to noxious pinch of the tail was suppressed by i.t. baclofen, suggesting that the doses of baclofen that were employed were sufficient to affect spinal pathways. However, the abdominal EMG response to expiratory threshold loading was unaffected by i.t. administration of either baclofen or codeine. These results indicate that neither baclofen nor codeine suppress cough *via* a spinal action and support the concept that the antitussive effect of these drugs is restricted to the brainstem.

Keywords: baclofen, codeine, airway protection, cough, intrathecal, antitussives, opioid, GABA-B receptor agonist

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INTRODUCTION

Cough is a protective behavior meant to remove irritant materials from the airways with explosive bursts of air and is characterized by a large and coordinated increase in activity in inspiratory and expiratory muscles (Ross et al., 1955; Widdicombe, 1980). However, pathological factors can modify respiratory function and increase the frequency and persistence of cough, which can lead to significant morbidity. As a result, antitussives are used to ameliorate symptoms of chronic cough

(Aylward et al., 1984). However, in a large number of human studies antitussives often lead to unpleasant side effects and there is a large demand for additional research on safer and more effective cough suppressants (Dicpinigaitis et al., 1997, 2014; Dicpinigaitis, 2009; Smith et al., 2012).

Antitussives are thought to inhibit cough by acting in the brainstem at a central site of action (Keller et al., 2017; Korpás and Tomori, 1979; Mutolo et al., 1985; Satia et al., 2016). These drugs work through a central mechanism by inhibiting a functionally identified segment of the brainstem control mechanism for cough termed a gate. The gate is proposed to control the expression of tracheobronchial (TB) cough. Given that laryngeal cough is relatively insensitive to suppression by intravenous (i.v.) codeine (Keller et al., 2017; Korpás and Tomori, 1979; Mutolo et al., 1985; Satia et al., 2016), the gating hypothesis does not extend to coughing induced from the larynx. However, cough from this organ may have a similar control mechanism, but if so, it is insensitive to antitussives. The gating mechanism is proposed to consist of specialized populations of neurons that directly excite the respiratory/cough pattern generator and modulate the expiratory motor activity related to cough (Bolser and Davenport, 2002, 2007; Bolser et al., 2006; Bolser, 2006). Specifically, there are interneurons located in the nucleus tractus solitarius (NTS) that affect afferent information, premotor inspiratory and expiratory neurons in the medulla, and expiratory and inspiratory motoneurons in the spinal cord (Bolser and Davenport, 2002). Intrathecal (i.t.) administered antitussives may, in part, have an effect on cough through the expiratory and inspiratory motoneurons *via* the spinal cord. This alternative route of administration could alleviate negative reported side effects.

While a brainstem site of action for antitussive drugs is generally accepted, there is no information regarding potential direct actions of these drugs on spinal circuits controlling chest wall and abdominal motor drive to respiratory muscles. Suppression of cough-related motor drive, especially to abdominal muscles, is a prominent feature of the effects of these drugs in animal models (Bolser, 1985; Poljacek et al., 1985, 2010; Bolser et al., 1993, 1997, 1999; Mutolo et al., 2008; Castillo and Pitts, 2013; Xu et al., 1985). We hypothesized that antitussive drugs would act, in part, to suppress abdominal motor drive during cough through an action in the spinal cord. We tested this hypothesis by administering two well-known cough suppressants, codeine and baclofen, *via* the i.t. route in a feline model of cough. Codeine was chosen because it is a standard cough suppressant drug in animal models and is one of the most widely prescribed antitussives in humans (Schappert, 1999). The GABA-B receptor agonist, baclofen, was chosen because it has antitussive activity in animal models (Bolser et al., 1993; Bolser et al., 1994; Canning et al., 2012) and humans (Dicpinigaitis et al., 1998). Further, baclofen was originally introduced in the 1960's to treat spasticity *via* an action through a GABA system potentially in the spinal cord (Bowery, 1982). While the central actions of baclofen in the brainstem was thoroughly studied in animal models (Dicpinigaitis et al., 1997; Bolser et al., 1999; Bolser and Davenport, 2002, 2007; Bolser, 2006; Canning et al., 2012; Korpás and Tomori, 1979), to our

knowledge a potential spinal action of this drug to suppress cough has never been studied.

MATERIALS AND METHODS

The experiments, procedures, and method of euthanasia described herein was approved by the University of Florida Animal Care and Use Committee and were performed in accordance with the *Guide for the Care and Use of Laboratory Animals*. Twenty-two (2.0–5.0 kg) spontaneously breathing cats were anesthetized with sodium pentobarbital (35 mg/kg, i.v.). Sixteen of 22 were male and six were female. End-tidal CO₂ (Datex Engstrom, *Capnomac Ultima*; ET_{CO2}) was monitored, and supplemental doses of anesthetic (0.1 mg/kg, i.v.) were administered when the ET_{CO2} dropped below 3.9%. Additionally, palpebral reflexes, jaw tone, and blood pressure [Becton Dickenson (B-D) – Model P23XL] were continuously monitored to maintain appropriate anesthesia levels of the animals. In the case of animal responsiveness to reflexes and/or jaw tone, the animal was administered supplemental doses of anesthetic and the experiment was re-tested before proceeding with experimental procedures. Catheters were placed in a femoral artery and vein for monitoring blood pressure, arterial gases (Abbott Labs, *I-stat*) and administering drugs, respectively. Atropine sulfate (0.1 mg/kg i.v.) was administered to block reflex tracheal secretions. This dose was informed by veterinarians and is consistent with doses of those used in veterinary clinical practice used to reduce reflex mucus secretion (Plumb, 2011). A three-way tracheal cannula was inserted through an incision at the fourth tracheal segment. Body temperature was maintained at 37 ± 1°C with a homeothermic blanket system (Harvard Apparatus).

Animal Preparation

An incision was made in the left forelimb. The brachial plexus was exposed and the costocervical, thyrocervical, and internal thoracic branches of the axillary artery were identified and clamped. A cannula was placed in the axillary artery and its tip moved to the vertebral arterial branch. Bipolar plastic-coated stainless steel wire electrodes were placed in the parasternal (PS), rectus abdominis, and transversus abdominis (TA) muscles according to the technique of Basmajian and Stecko (Basmajian and Stecko, 1962). Electrodes were placed in the PS muscle at T₃. The rectus abdominis muscle electrodes were placed through a small incision in the skin, approximately 7 cm caudal to the xiphoid process and 1 cm lateral to the midline.

Recording Procedures

Electrodes were placed in TA muscles through an incision in the left lower abdominal skin, midway between the midline and the axillary line. Electromyograms (EMGs) from these muscles were amplified (Grass, Model *P511*), band-pass filtered (0.1–5.0 kHz), monitored on an oscilloscope, and integrated with a resistance-capacitance circuit (100-ms time constant; Cambridge Electronic Design, 1,401 Mrk II) and recorded using Cambridge Electronic Design, Spike II software.

For i.t. injections, animals were placed prone in a stereotaxic frame with their head elevated above the spinal column and remained in the frame for the duration of the experiment. An incision was made just over the lumbosacral space. An 18-gauge, 3-inch spinal needle was placed just caudal to L7 and advanced through the dura. The stylet was examined for flow of cerebrospinal fluid (CSF) to confirm subdural placement. A 19-gauge cannula (B-Braun Medical Inc.,—Open-tip) was advanced approximately 5 cm through the stylet into the subdural space for the i.t. administration of vehicle, baclofen, or codeine. Placement of the cannula was verified postmortem.

Cough was defined as an inspiratory-related burst of EMG activity in the PS muscle immediately followed by a burst of EMG activity in the abdominal muscles. Cough was elicited by mechanical stimulation of the intrathoracic trachea with a small length of polyethylene tubing (PE90) into the intrathoracic trachea *via* the tracheal cannula. This catheter was inserted into the trachea for 10 s (rotated at approximately 2 Hz) to elicit repetitive coughing. Breathing was monitored by the EMG activity of the PS, rectus abdominis, and TA muscles and continuous ET_{CO2} monitoring.

Protocol

A series of 10 s mechanical stimuli was applied to the intrathoracic airways were performed with a total of 1 min elapsed time between stimulus trials to establish a stable baseline for TB cough. The antitussive activity of baclofen was evaluated from cumulative dose-response relationships (10 and 100 $\mu\text{g}/\text{kg}$) after i.t. and successive intra-arterial (i.a.) (10 and 100 $\mu\text{g}/\text{kg}$) administration. The antitussive activity of codeine was also evaluated at doses of 30 $\mu\text{g}/\text{kg}$ for i.t. and 30 $\mu\text{g}/\text{kg}$ i.a. administration in a separate group of animals. Additionally, vehicle (artificial CSF for i.t. and physiological saline for i.a.) was administered. The highest doses chosen for each drug represented ED₅₀₋₇₀ values established in this model in a previous study (Bolser et al., 1999).

Stimulation Protocol for Baclofen and Codeine

Following the i.t. doses, 5–7 min were allowed to elapse before trial commencement. Stimulus trials were applied at 1 min intervals for a total of five stimulus trials between i.t. doses. Approximately 60 s following the cough trial series, a non-rebreathing valve was attached to the tracheal cannula. An expiratory load of 15 cm H₂O was applied by attaching a hose to the expiratory port of the valve and submerging the end of the hose in a reservoir of water. Each load was 1 min in duration. Approximately 60 s following the expiratory load, a noxious mechanical stimulus was applied to the base of the tail by clamping down with a hemostat for a duration of 60 s. The hemostat was rotated in the horizontal plane by approximately 45 degrees at a frequency of 0.5 Hz for the duration of the trial. After the completion of the noxious stimulus, approximately 1 min lapsed before beginning the next i.t. dose. Once the i.t. doses had been administered, the i.a. protocol commenced. Following the i.a. doses, 5–7 min were allowed to elapse before trial commencement. Stimulus trials for TB cough, expiratory load, and noxious mechanical stimulus were applied similarly

as in the i.t. administration. Codeine trials occurred similarly. The noxious mechanical stimulus was not applied in the codeine protocol because we were aware of codeine's effective dosages.

Compounds

Compounds used in this study include atropine sulfate (Sigma Chemical Co., St. Louis, MO, United States), artificial Cerebral Spinal Fluid (CSF) (Harvard Apparatus, Holliston, MA, United States), codeine phosphate (Sigma Chemical Co., St. Louis, MO, United States), and baclofen (Research Biochemicals, Natick, MA, United States). Baclofen (i.a.), codeine (i.a.), and atropine were dissolved in physiological saline. Artificial CSF was employed as the vehicle for i.t. administration of baclofen and codeine.

Statistics

The data were analyzed using SigmaPlot 13.0 (Systat Software, Inc.). The data are expressed as mean \pm s.e. mean. A student's *t*-test, one-way ANOVA, or two-way ANOVA was used to evaluate differences between means. If a normality test (Shapiro–Wilk, $p < 0.05$) was failed, an ANOVA on ranks was conducted. Differences between means were considered significant if $p < 0.05$, and were followed by *post hoc* pair wise comparisons (Holm–Sidak method if normality was not violated and Dunn's test if an ANOVA on ranks test was conducted). The EMG amplitudes of the three largest bursts during each expiratory load were averaged to obtain a single value for each load. The EMG burst amplitudes during expiratory loading, tail pinch and coughing were expressed as a percentage of the average of the pre-vehicle amplitudes. EMG activity during the tail pinch stimulus was analyzed by calculating the area of abdominal EMG activity during the 60-second stimulus then comparing the differences in area. Cardiorespiratory results for both baclofen and codeine are discussed in **Table 1**.

RESULTS

There were 22 animals used for this study. A total of 4,015 TB coughs were evaluated in six animals who received baclofen, five animals who received codeine, and four control animals (15 total). All six animals that were administered baclofen also were challenged with expiratory threshold loading and noxious mechanical stimulation of the tail. Seven additional animals were evaluated with noxious stimulation of the tail but were not challenged with TB cough (one administered baclofen, six administered vehicle). For codeine administration, four of five animals that were challenged with TB cough were also challenged with expiratory threshold loading and none were challenged with noxious mechanical stimulation of the tail.

Baclofen

Abdominal and PS muscle EMGs were strongly activated during repetitive coughing (**Figure 1**). A one-way ANOVA revealed that i.t. baclofen had no significant effect on the number of TB coughs (**Figure 2**), at 10 $\mu\text{g}/\text{kg}$ ($p = 0.97$) or 100 $\mu\text{g}/\text{kg}$ ($p = 0.82$), relative to vehicle for the baclofen group. Further, i.t. baclofen

TABLE 1 | Influence of baclofen and codeine on cough and cardiorespiratory parameters.

	Baclofen				
	Vehicle	10 $\mu\text{g}/\text{kg}$ i.t.	100 $\mu\text{g}/\text{kg}$ i.t.	10 $\mu\text{g}/\text{kg}$ i.a.	100 $\mu\text{g}/\text{kg}$ i.a.
CT _I	0.74 + 0.07 s	0.67 + 0.14 s	0.67 + 0.14 s	0.65 + 0.18 s	0.75 + 0.10 s
CT _E	0.91 + 0.06 s	0.84 + 0.17 s	0.80 + 0.17 s	0.81 + 0.16 s	0.97 + 0.11 s
CT _{TOT}	1.64 + 0.38 s	1.51 + 0.31 s	1.47 + 0.31 s	1.47 + 0.33 s	1.72 + 0.21 s
BP	143.67 + 5.80	135.0 + 5.47	124.0 + 6.40	126.50 + 5.92	131.28 + 6.55
PS _{AMP}	100.0	100 + 6	95 + 5	93 + 7	104 + 14
T _I	1.61 + 0.26	1.19 + 0.20	1.32 + 0.26	1.39 + 0.14	1.30 + 0.26
T _E	1.35 + 0.20	1.47 + 0.25	1.62 + 0.24	1.50 + 0.26	1.45 + 0.20
pH		7.38 + 0.02		7.39 + 0.01	
pCO ₂		28.9 + 0.50		29.6 + 0.90	
pO ₂		101.7 + 1.90		100.2 + 1.90	
ETCO ₂		31.7 + 1.40		29.4 + 0.70	
HR _{Min}	207.33 + 8.03	210.67 + 9.62	211.67 + 9.65	218.33 + 8.85	222.33 + 8.86
RR _{Min}	22.5 + 2.16	23.5 + 2.50	23.2 + 2.95	24.0 + 1.55	25.5 + 2.85

	Codeine		
	Vehicle	30 $\mu\text{g}/\text{kg}$ i.t.	30 $\mu\text{g}/\text{kg}$ i.a.
CT _I	0.70 + 0.10 s	0.61 + 0.14 s	0.83 + 0.27 s
CT _E	1.04 + 0.18 s	0.85 + 0.17 s	1.27 + 0.56 s
CT _{TOT}	1.74 + 0.55 s	1.45 + 0.36 s	2.09 + 0.84 s
CT _{TOT}	1.74 + 0.55 s	1.45 + 0.36 s	2.09 + 0.84 s
BP	139.80 + 11.10	133.80 + 15.50	130.40 + 18.90
PS _{AMP}	1.0	94 + 5	90 + 8
T _I	1.49 + 0.44	1.28 + 0.32	1.45 + 0.79
T _E	1.42 + 0.58	1.32 + 0.42	1.51 + 0.36
Ph		7.39 + 0.01	7.38 + 0.01
pCO ₂		32.0 + 1.49	30.4 + 2.30
pO ₂		103.4 + 2.84	104.2 + 8.55
ETCO ₂		31.0 + 1.48	32.0 + 1.67
HR _{Min}	225.6 + 9.02	226.80 + 8.98	280.32 + 13.50
RR _{Min}	24.0 + 3.29	25.8 + 2.62	17.8 + 2.69

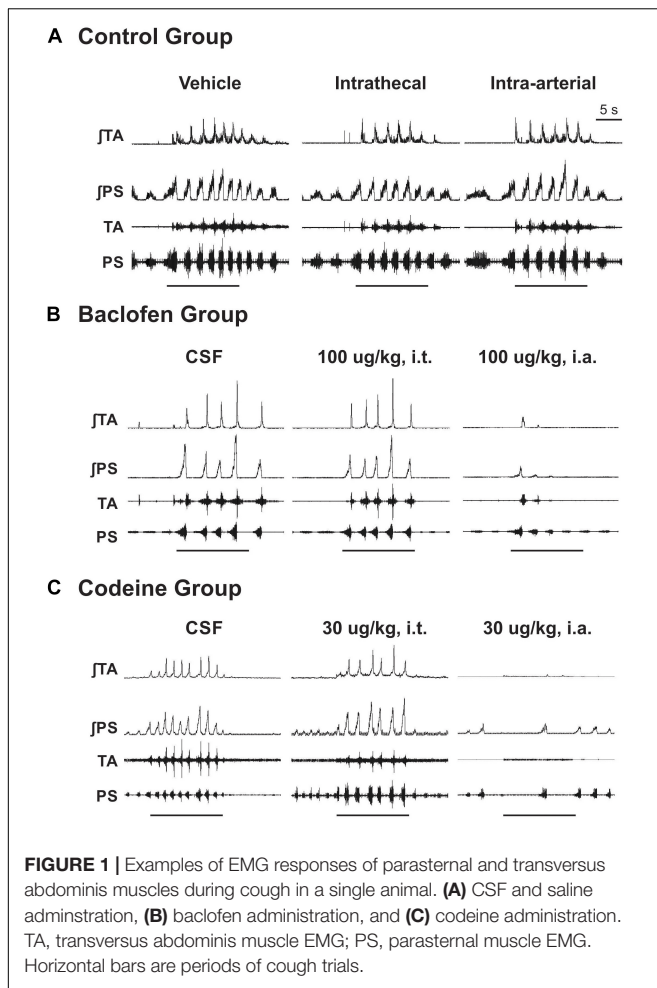
A one-way ANOVA was conducted for the cough phase analysis. Cough phases were not significantly different for baclofen or codeine at any dose. Blood pressure (BP), parasternal amplitudes (PS_{AMP}), respiratory rate (RR), heart rate (HR), total inspiratory (T_I) time, and total expiratory (T_E) time were analyzed using a one-way ANOVA. No significant differences were observed at any dose. pH levels, pCO₂, pO₂, and ETCO₂ for each administration route (intrathecal and intra-arterial) were subjected to a Student's *t*-test. No significant differences were revealed. The number of animals for baclofen and codeine-treated animals were 6 and 5, respectively. Data are expressed as mean \pm s.e. mean.

did not significantly affect abdominal EMG amplitude during TB cough (**Figure 2**) at either dose (10 $\mu\text{g}/\text{kg}$, $p = 0.71$ or 100 $\mu\text{g}/\text{kg}$, $p = 0.53$) or PS EMG amplitude at either dose (**Figure 2**; 10 $\mu\text{g}/\text{kg}$, $p = 0.75$ or 100 $\mu\text{g}/\text{kg}$, $p = 0.86$). There were no significant effects of i.a. baclofen at the 10 $\mu\text{g}/\text{kg}$ dose (**Figure 2**; $p = 0.16$ on abdominal or PS (**Figure 2**; $p = 0.76$) EMG activity relative to vehicle. A two-way ANOVA was conducted to determine if differences existed between the animals who received baclofen and the control group (animals who received CSF i.t. and saline i.a.) at different doses for number of coughs, abdominal, and PS EMG magnitudes. Results revealed a Group \times Dose interaction for the PS EMG condition ($p = 0.007$). A *post hoc* comparison determined that the baclofen group had reduced magnitudes of the PS muscles at the 100 $\mu\text{g}/\text{kg}$ i.a. dose when compared to the control group (**Figure 2**). Additionally, results indicated that the baclofen group did have significantly reduced magnitudes of the TA ($p = 0.013$) and fewer number of coughs ($p = 0.008$) during TB

cough trials, compared to the control group. i.a. administration of baclofen did significantly decrease abdominal ($p < 0.001$) and PS ($p < 0.001$) EMG activity at the 100 $\mu\text{g}/\text{kg}$ dose when compared to vehicle. Further, i.a. administration of baclofen decreased TB cough number at the 10 $\mu\text{g}/\text{kg}$ dose ($p = 0.049$) and the 100 $\mu\text{g}/\text{kg}$ dose ($p = 0.004$), relative to vehicle.

A one-way ANOVA was conducted for a cough phase analysis at vehicle and each dose of baclofen. Values for inspiratory, expiratory and total cough phase duration means and their s.e.m.'s are reported in **Table 1** (Wang et al., 2009). Results indicated that there were no significant differences for i.t. or i.a. administration at any dose ($p = 0.095$). During expiratory threshold loading, there was no significant difference in the magnitude of abdominal EMG burst activity during the i.t. or i.a. administration of baclofen at any dose ($p = 0.93$; **Figure 3**).

A two-way ANOVA was conducted to determine if differences existed between the animals who received baclofen and the



control group (animals who received CSF i.t. and saline i.a.) at different doses for noxious somatic stimulation (tail pinch stimulus). Results indicated that differences existed between the control and baclofen group, $p < 0.001$. For animals who received baclofen, a one-way ANOVA revealed i.t. and i.a. administration of baclofen did significantly reduce abdominal motor activity induced by a noxious somatic stimulus applied to the base of the tail at the 10 $\mu\text{g}/\text{kg}$ ($n = 7$; i.t. $p = 0.005$; i.a. $p < 0.001$; **Figure 4**) and 100 $\mu\text{g}/\text{kg}$ doses ($n = 7$, i.t. and i.a. $p < 0.001$; **Figure 4**) when compared to vehicle administration. The suppressive effect of baclofen on abdominal EMG responses to noxious somatic stimulation indicates that the i.t. and i.a. doses of baclofen chosen were effective when compared to a vehicle intervention ($n = 7$; $p < 0.001$, i.t. and $p < 0.001$ i.a.; **Figure 4**).

Codeine

A one-way ANOVA revealed that i.t. administration had no significant effect (30 $\mu\text{g}/\text{kg}$, $n = 5$; $p = 0.61$) on TB cough, relative to vehicle. i.t. administration of codeine did not significantly affect abdominal ($n = 5$; $p = 0.60$) or PS ($n = 5$; $p = 0.19$) EMG magnitudes at 30 $\mu\text{g}/\text{kg}$. Conversely, a two-way ANOVA was conducted to determine if differences existed between the animals who received codeine and the control group (animals

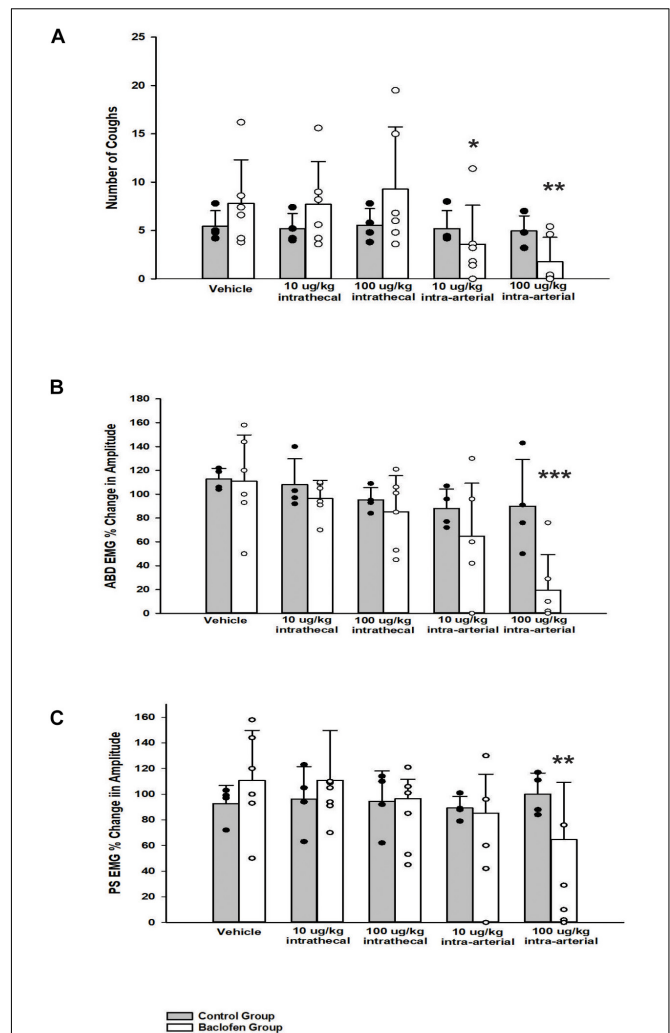
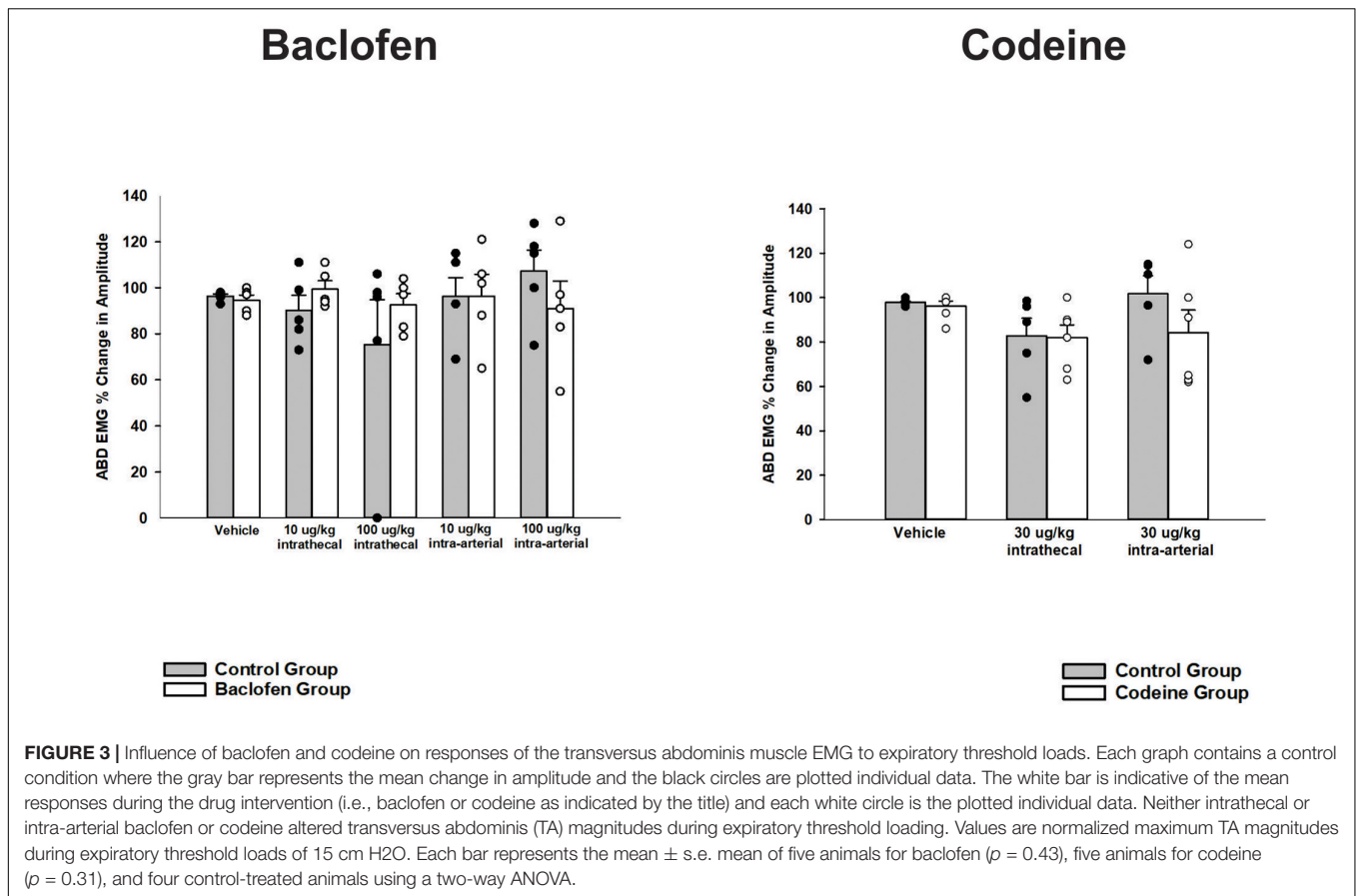


FIGURE 2 | Influence of baclofen on (A) the number of tracheobronchial cough, (B) the magnitude of transversus abdominis muscle during tracheobronchial cough, and (C) the magnitude of parasternal muscle during tracheobronchial cough. A two-way ANOVA revealed a significant difference between the control and baclofen group (A) $p = 0.008$, (B) $p = 0.013$, and (C) $p = 0.007$. The baclofen group had fewer number of coughs and smaller magnitudes of the transversus abdominis and parasternal muscles during tracheobronchial cough when compared to the control group. Specifically, a one-way ANOVA revealed the baclofen group had a significant reduction in: number of coughs at the 10 $\mu\text{g}/\text{kg}$ i.a. ($p = 0.049$) and the 100 $\mu\text{g}/\text{kg}$ i.a. dose ($p = 0.004$), magnitude of the transversus abdominis muscle at the 100 $\mu\text{g}/\text{kg}$ i.a. dose ($p < 0.001$), and magnitude of the parasternal muscle at the 100 $\mu\text{g}/\text{kg}$ i.a. dose ($p = 0.001$). Each bar represents the mean \pm s.e.m. of six animals who received baclofen (Baclofen Group) and 4 control animals (Control Group). Cough numbers were normalized by totaling the number of coughs and dividing by the number of trials. Amplitudes were normalized by magnitudes observed in the vehicle period. An asterisk (* $p < 0.05$, ** $p < 0.005$, *** $p < 0.001$) indicates a significant reduction in cough and amplitude relative to vehicle and the Control Group.

who received both CSF i.t. and saline i.a.) at different doses for number of coughs, abdominal, and PS EMG magnitudes. Results revealed a Group \times Dose interaction for number of coughs ($p < 0.001$), magnitudes of the TA ($p = 0.030$), and PS



($p < 0.001$) muscles. A *post hoc* pairwise comparison indicated that the codeine group did have significantly reduced number of coughs ($p < 0.001$), magnitudes of the TA ($p < 0.001$), and PS ($p < 0.001$) muscles during TB cough trials at the 30 $\mu\text{g}/\text{kg}$ i.a. dose, compared to vehicle and the control group (Figure 5).

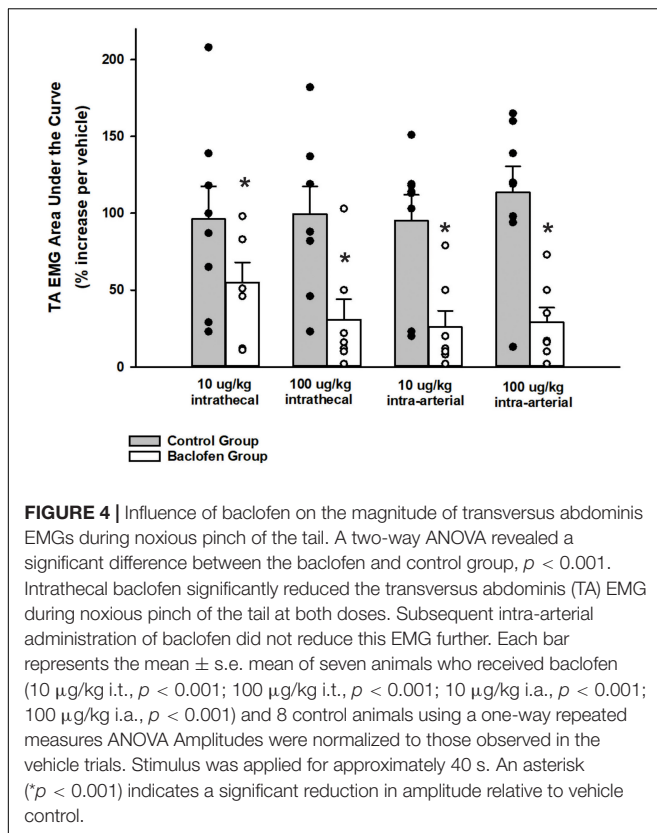
A one-way ANOVA was conducted for a cough phase analysis at vehicle and each dose of codeine. Values for inspiratory, expiratory and total cough phase duration means and their s.e.m.'s are reported in Table 1 (Wang et al., 2009). Results indicated there were no significant differences for codeine on cough phase durations when administered i.t. or i.a. ($n = 5$; $p = 0.303$). During expiratory threshold loading, there was no significant difference in abdominal EMG burst activity during the i.t. or i.a. administration of codeine at any dose ($n = 4$; $p = 0.31$; Figure 3).

DISCUSSION

The main findings of this study are that i.t. administration of baclofen and codeine did not significantly inhibit TB cough but i.a. delivery of these drugs reduced both cough number and the magnitude of expiratory muscle EMGs during coughing. Neither baclofen nor codeine suppressed abdominal motor drive during expiratory threshold loads. However, the abdominal EMG

response to a noxious stimulus applied to the base of the tail was significantly inhibited by i.t. administration of baclofen.

Baclofen has been shown to suppress coughing in animals and humans (Bolser et al., 1994, 1999; Canning et al., 2012; Dicipinigaitis et al., 1997) and codeine also suppresses cough in animal models (Bolser et al., 1995; Chou and Wang, 1975; May and Widdicombe, 1954), but the antitussive activity of codeine in humans has been questioned recently (Bolser and Davenport, 2007; Smith et al., 2006). Both drugs have central antitussive properties that reduce the number and magnitude of coughing in animal studies (Bolser et al., 1993, 1994, 1995; Bolser and DeGennaro, 1994; Simera et al., 2010). We and others demonstrated that effective dose ratios generated from i.v. and i.a. (vertebral artery) dose-response relationships can differentiate between the cough suppressant actions of drugs that have solely a peripheral site of action from those with a central component (Bolser et al., 1995; Chou and Wang, 1975). Doses of centrally active antitussive drugs when administered *via* the brainstem circulation are comparatively low relative to i.v. administration because of direct delivery to their site of action (Bolser et al., 1995). Our prior work showed that baclofen and codeine have high effective dose ratios (Bolser et al., 1993, 1994, 1995) in the cat and we suggested that the brainstem is a major site of action to suppress cough for these drugs. The evidence for central antitussive actions of these drugs also includes studies that utilized direct administration



to the brainstem by microinjection or intracerebroventricular administration (Mutolo et al., 1985, 2008; Ohi et al., 2005; Poliacek et al., 2010, 2012; Mutolo, 2017; Poliacek et al., 2017). The results of these studies convincingly attributed the actions of these drugs to selected areas of the brainstem. On balance, the evidence supports actions of these drugs at multiple sites in the brainstem. As such, administration of baclofen, codeine or other centrally-action cough suppressant drugs by vascular routes would be expected to affect the excitability of cough-related neurons in several areas of the brainstem. However, these drugs also enter the spinal cord after systemic administration. Potential actions of these cough suppressant drugs in the spinal cord would be expected to be limited to suppression of motor drive to respiratory muscles resulting in weaker coughs. The number of coughs and their temporal features are regulated by brainstem neuronal circuits (Shannon et al., 1996, 1998, 2000, 2004a; Bongianini et al., 1998; Baekey et al., 2003; Shannon et al., 2004b). Our present results do not support a spinal action on cough-related motor pathways by codeine or baclofen. While peripheral actions of these drugs on cough following systemic administration are possible (Gruhzit, 1957), it is likely that the major actions of these drugs on motor features of coughing are restricted to the brainstem, at least in animal models. We cannot rule out actions of these drugs on suprapontine pathways that participate in coughing (McGovern et al., 2015a; McGovern et al., 2015b). Indeed, suprapontine areas present strong contributions to the genesis of urge-to-cough in humans (Mazzone et al., 2007, 2009, 2011, 2013; Mazzone et al., 2015).

When administered to the brainstem circulation, baclofen and codeine significantly inhibited TB cough. These findings are consistent with the findings of Bolser et al. (1993), Bolser and DeGennaro (1994), Bolser et al. (1994), and Canning et al. (2012). For both baclofen and codeine, cough number decreased by more than 60% when administered intra-arterially, relative to vehicle and control. Cough-related abdominal muscle EMG activity was depressed by i.a. administration of either baclofen or codeine. PS inspiratory muscle activity was significantly depressed during cough at the highest dose of baclofen (100 $\mu\text{g}/\text{kg}$ i.a.) and codeine administration (30 $\mu\text{g}/\text{kg}$ i.a.). These findings represent a significant departure from the results of previous studies in our laboratory that diaphragm muscle EMG activity during TB coughing was not affected by i.a. administration of codeine at this dose (Castillo and Pitts, 2013; Xu et al., 1985). For baclofen, previous work has shown that doses up to 30 mg/kg, i.a. had no effect on diaphragm motor drive during cough in the cat (Bolser et al., 1994). In these previous studies, PS inspiratory muscle EMG activity was not recorded. The present study shows that a higher i.a. dose of this drug does result in suppression of inspiratory motor drive to PS spinal inspiratory motoneuron pools during cough. This motoneuron pool can be subject to differential regulation during non-breathing postural behaviors in humans relative to other inspiratory muscles (Butler et al., 2014). The extent to which pharmacological interventions can have differential actions on diaphragm and PS motoneuron activity during coughing is not known.

Baclofen has been historically used to treat spasticity in spinal cord injury and cerebral palsy patients *via* i.t. administration (Albright et al., 1991; Penn et al., 1989). The lack of effect of this drug on cough-related or expiratory load induced increases in abdominal motor drive was somewhat surprising. However, the fact that the abdominal muscle EMG response to noxious pinch of the tail was suppressed by i.t. baclofen indicates that the doses selected for the study were sufficient to decrease non-cough motor responses controlled by spinal circuitry (Kato et al., 1978; Straube et al., 2014). Therefore, the lack of effect of baclofen on abdominal cough-related EMG magnitudes are unlikely to be explained by subthreshold i.t. dosing.

Premotor neurons to spinal abdominal muscle motoneurons are located in the caudal ventral respiratory column in the cat, which corresponds to the nucleus retroambigualis (Iscoe, 1998). We have reported that microinjection of codeine into this area did not suppress abdominal motor drive that was induced by microinjection of the excitatory amino acid agonist, D,L-homocysteic acid, into the same location (Poliacek et al., 2010). In the current study, we found that abdominal motor drive that was induced by expiratory threshold loads was not affected by either i.t. or i.a. baclofen or codeine, although i.a. administration of these drugs did alter cough-related abdominal motor drive. Increases in abdominal motor drive due to respiratory loading are due to decreased pulmonary volume-related feedback and prolongation of the expiratory phase of breathing, leading to a higher peak drive of expiratory premotor neurons in the caudal medulla (Bajic et al., 1992). These findings support our previous conclusion (Poliacek et al., 2010) that suppression of abdominal motor drive during cough by centrally-acting antitussive drugs

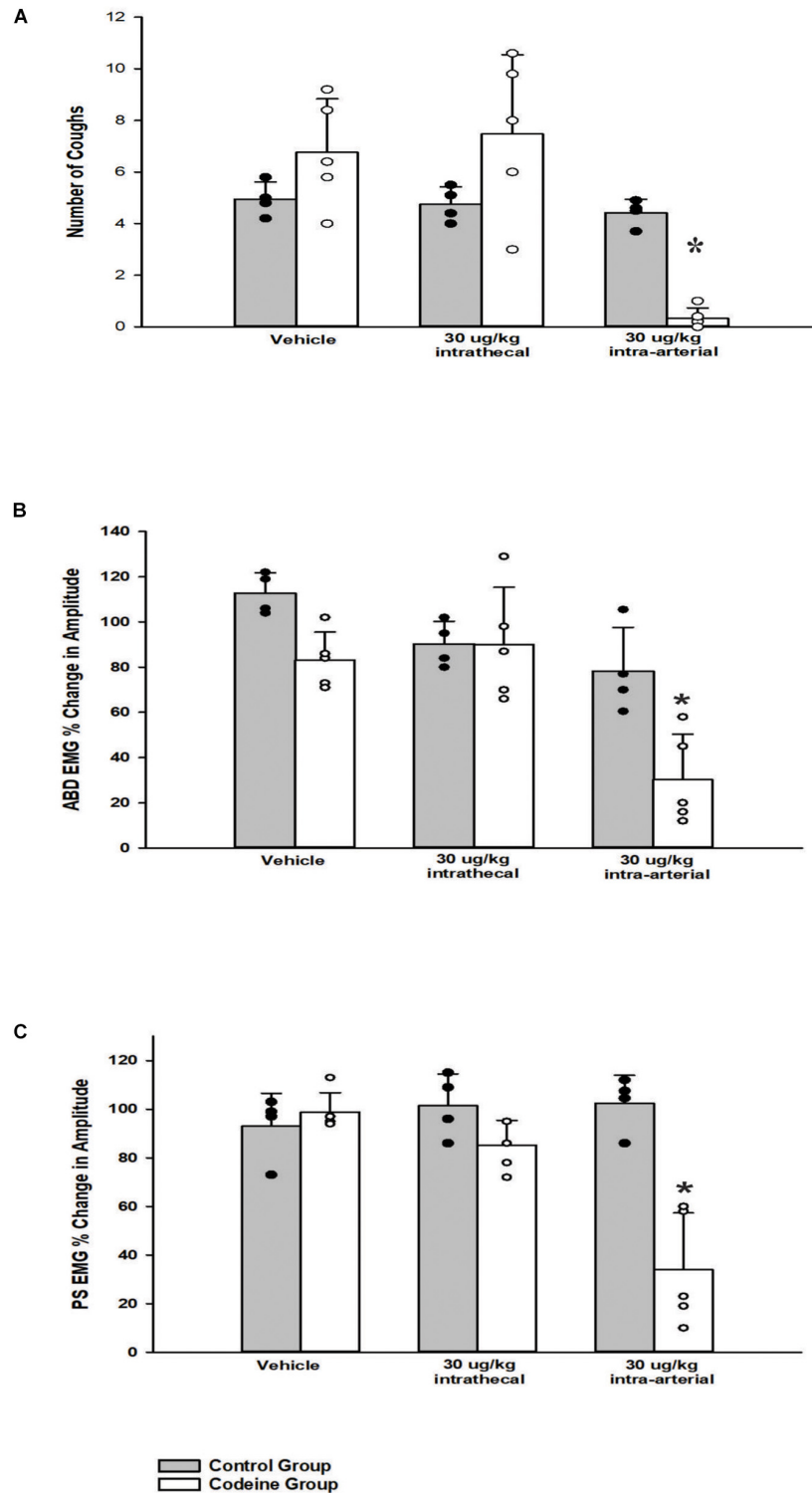


FIGURE 5 | Influence of codeine on **(A)** the number of tracheobronchial cough, **(B)** the magnitude of transversus abdominis muscle during tracheobronchial cough, and **(C)** the magnitude of parasternal muscle during tracheobronchial cough. A two-way ANOVA revealed a Group x Dose interaction for **(A)** $p < 0.001$, **(B)** $p = 0.030$, and **(C)** $p < 0.001$. The codeine group had fewer number of coughs and smaller magnitudes of the transversus abdominis and parasternal muscles during tracheobronchial cough at the 30 $\mu\text{g}/\text{kg}$ i.a. dose, **(A)** $p < 0.001$, **(B)** $p < 0.001$, and **(C)** $p < 0.001$. Each bar represents the mean \pm s.e.m. of five animals who received codeine (Codeine Group) and four control animals (Control Group). Cough numbers were normalized by totaling the number of coughs and dividing by the number of trials. Amplitudes were normalized by magnitudes observed in the vehicle period. An asterisk (* $p < 0.001$) indicates a significant reduction in amplitude in cough and amplitude relative to vehicle and the Control Group.

is likely to be due to actions on neurons other than expiratory premotor neurons in the medulla. We also suggest that activation of expiratory spinal motor pathways by perturbation of slowly adapting receptor activity (*via* respiratory loading) is due to brainstem and spinal pathways that are relatively insensitive to cough suppressants.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by the University of Florida Animal Care and Use Committee.

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AUTHOR CONTRIBUTIONS

WO analyzed and interpreted data with DB, prepared and edited the manuscript, prepared the graphs and table, and submitted the manuscript for peer review. MR conducted experiments, analyzed data, collected data, and assisted with manuscript preparation. FG, CW, and JH assisted with conducting experiments and data collection. DB is the senior author who conceived the protocol, assisted with interpretation of data, and manuscript preparation. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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