Microinjection of codeine into the region of the caudal ventral respiratory column suppresses cough in anesthetized cats

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Poliacek I, Wang C, Corrie LW, Rose MJ, Bolser DC. Microinjection of codeine into the region of the caudal ventral respiratory column suppresses cough in anesthetized cats. J Appl Physiol 108: 858–865, 2010. First published January 21, 2010; doi:10.1152/japplphysiol.00783.2009.—We investigated the influence of microinjection of codeine into the caudal ventral respiratory column (cVRC) on the cough reflex. Experiments were performed on 36 anesthetized spontaneously breathing cats. Electromyograms (EMGs) were recorded bilaterally from inspiratory parasternal and expiratory transversus abdominis (ABD) muscles and unilaterally from laryngeal posterior cricoarytenoid and thyroarytenoid muscles. Repetitive coughing was elicited by mechanical stimulation of the intrathoracic airways. The unilateral microinjection of codeine (3.3 mM, 20–32 nl) in the cVRC reduced cough number by 29% (P < 0.01) and expiratory cough amplitudes of esophageal pressure by 33% (P < 0.05) as well as both ipsilateral and contralateral ABD EMGs by 35% and 48% (P < 0.01 and P < 0.01, respectively). No cough depression was observed after microinjections of vehicle. There was no significant effect of microinjection of codeine in the cVRC (3.3 mM, 30–40 nl) on ABD activity induced by a microinjection of D,L-homocysteic acid (30 mM, 27–40 nl) in the same location. However, a cumulative dose of codeine (0.1 mg/kg, 330 nmol/kg) applied into the brain stem circulation through the vertebral artery reduced the ABD motor response to cVRC D,L-homocysteic acid microinjection (30 mM, 28–32 nl) by 47% (P < 0.01). These results suggest that 1) codeine can act within the cVRC to suppress cough and 2) expiratory premotoneurons within the cVRC are relatively insensitive to this opioid.

Cough is one of the most widely prescribed drugs to treat cough. Several studies (18, 20, 43, 56) have reported reduced coughing of different etiologies by codeine. However, more recent studies (19, 23, 61) have questioned the efficacy of this drug in humans with airway pathology. The treatment of cough by codeine and other antitussives is based on the reduced severity of cough bouts or attacks representing a reduced number of individual coughs and diminished expiratory cough efforts (5, 7, 11, 35, 38, 49).

Codeine acts to reduce coughing on the central neuronal cough circuitry within the brain stem (6, 36). Some authors (50) have proposed the site of the antitussive action of codeine to be within the nucleus tractus solitarii (NTS). The NTS is a primary projection site of vagal afferents including cough-related afferents and the location of second-order interneurons of the cough reflex arc (31, 40). However, older microinjection experiments (37) used doses of codeine (0.01 mg) that were unlikely to be restricted to the immediate region of the injection site (47, 48). Recent experiments on rabbits are consistent with the antitussive effects of central antitussives within the NTS (45); however, these findings do not exclude that the cough-related structures within other brain stem areas are sensitive to these drugs as well.

It is generally accepted that the cough motor pattern is produced by a common respiratory/cough central pattern generator (R/C CPG) (29, 57, 58). The second-order cough interneurons within the NTS (excited by cough-related stimulation) provide (directly or indirectly) an excitatory drive to the R/C CPG to reconfigure it for the generation of a cough motor pattern. However, analysis of the effects of codeine on the cough motor pattern does not support the NTS as an exclusive site of action for this drug (7, 9, 10). Similarly, it is unlikely that suppression of some components of the core R/C CPG may account for the pattern of cough suppression observed in animal experiments (9, 10).

Because codeine and other central antitussives have a pronounced effect on expiratory cough amplitudes, it is reasonable to hypothesize a depressive effect of these drugs on expiratory premotoneurons within caudal ventral respiratory column (cVRC). Moreover, we (54) have recently identified a cough suppressant neuronal mechanism (termed a cough suppressor) located within the area of the cVRC. This cough suppressor could take part in the cough reduction induced by central antitussive drugs. To examine these possibilities, we microinjected codeine into the area of the cVRC where D,L-homocysteic acid (DLH) inhibited cough as well (54) and studied its effect on coughing. We also microinjected DLH into the same location to examine the effect of local or intra-arterial codeine on abdominal (ABD) discharge induced by the excitation of expiratory premotoneurons by DLH.

We hypothesized that codeine administered locally in the cVRC would suppress the expiratory component of cough. We also speculated that ABD activity produced by DLH stimulation of expiratory premotoneurons within the cVRC would be suppressed both by the intra-arterial and local administration of codeine.

MATERIALS AND METHODS

Experiments were performed on 36 female cats (3.65 ± 0.09 kg) under 3 different protocols [7 control animals were used from our previous study (54)]. All animals were anesthetized with pentobarbital sodium (35 mg/kg iv), and supplementary doses were administered (1–3 mg/kg iv) as needed. Atropine (0.1 mg/kg iv) was given at the beginning of the experiment to reduce secretions. The trachea and femoral artery and vein were cannulated. A balloon catheter was inserted into the esophagus for the measurement of esophageal pressure (EP). In animals under the “intra-arterial injection” protocol a cannula was introduced into the left brachial artery, and the tip was
positioned near the origin of the vertebral artery. All other branches of the subclavian artery in the region were clamped. All animals were allowed to spontaneously breathe a gas mixture of 40% oxygen-balance nitrogen. Arterial blood pressure (BP), EP, end-tidal CO$_2$ (ETCO$_2$), and body temperature were continuously monitored. Body temperature was controlled by a heating pad and maintained at 37.5 ± 0.5°C. Periodically, samples of arterial blood were removed for blood gas and pH analysis. All procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Florida.

Electromyograms (EMGs) of respiratory muscles were recorded with bipolar insulated fine wire electrodes using the technique of Basmajian and Stecko (3). EMGs were recorded bilaterally from the expiratory transversus ABD muscles and inspiratory parasternal (PS) muscles and unilaterally from the laryngeal abductor posterior cricoarytenoid (PCA) and laryngeal adductor thyroarytenoid (ThAr) muscles. Details of the placement of these electrodes were presented in our previous report (54).

Animals were placed prone in a stereotaxic frame, and the dorsal surface of the medulla was exposed. The surface of the brain stem was covered with warm paraffin oil. Microinjections of codeine (3.3 mM, Sigma-Aldrich) and/or the excitatory amino acid agonist DLH (30 mM, Sigma-Aldrich) were performed. Both drugs were dissolved in artificial cerebrospinal fluid (aCSF). Single or three-barrel glass micropipettes (tip diameter: 4 – 40 μm) and also composite three-barrel micropipettes with a carbon filament microelectrode were used for pressure microinjections of the solutions. The composite micropipettes allowed the recording of expiratory neuronal activities for the physiological confirmation of the micropipette tip location near to the cVRC. The tip of the micropipette/microelectrode was positioned under stereotaxic control into the region of the cVRC (1.8 – 3.5 mm caudal to the obex, 3 – 3.2 mm lateral to the midline, and 2.9 – 4.4 mm below the dorsal medullary surface). The injected volume was monitored by observation of movement of the meniscus in the micropipette barrel with a microscope. Injection sites were labeled by fluorescent latex beads as previously reported (Fig. 3A in Ref. 54). When composite micropipettes were used, we assumed that the microinjections were placed within the cVRC area only when expiratory multi-unit activity had been recorded. The positions of the micropipette tips were confirmed by the occurrence of a labeled spot in or near the caudal retroambigual nucleus (Fig. 1). In addition, we ran the protocols with DLH microinjections only when reproducible increases in ABD activity in response to DLH were induced (12, 54).

Tracheobronchial cough was elicited by mechanical stimulation of the intrathoracic airways with a thin polyethylene catheter or a custom mechanical stimulation device composed of one to three nylon fiber loops. This stimulator was inserted into the trachea (moved and rotated at a frequency of 1–2 Hz) for periods of 10 or 20 s to elicit repetitive coughing. Cough was defined by a large burst of inspiratory-related PS EMG activity immediately followed by a burst of expiratory ABD EMG activity and by a related inspiratory-expiratory waveform of EP. These criteria separated cough from other airway defensive behaviors such as augmented breath and mainly from the expiration reflex that is inducible from the trachea as well (55).

All EMGs were amplified, filtered (300 – 5,000 Hz), rectified, and integrated (time constant: 200 ms). The number of coughs in response to mechanical stimulation of the trachea [cough number (CN); average number of coughs per 10 s of stimulation], amplitudes of PS, ABD, and laryngeal muscle EMG moving averages and amplitudes of EP during the appropriate phases of cough and breathing, respiratory rate (RR), duration of inspiratory and expiratory phases of breathing (T$_I$ and T$_E$, respectively) and cough (CT$_I$ and CT$_E$), BP, heart rate, and ETCO$_2$ were analyzed in control, preinjection, and postinjection periods. The cough inspiratory phase was defined as the period from the onset of PS EMG activity until its maximum during cough. The cough expiratory phase was defined as the interval from the maximum of PS activity to the onset of the next PS EMG burst (7). In addition, we analyzed the duration of ABD EMG activity during the cough reflex. The activities of PCA and ThAr were analyzed separately in each of the four laryngeal cough phases (53) in accordance with our recent study (54).

When cough was suppressed by a microinjection, we frequently observed large inspiratory efforts in response to tracheobronchial stimulation that were not accompanied by expulsive activity. These inspirations may represent abrogated cough responses that do not include the expiratory cough component. We analyzed the spatiotemporal parameters of all enhanced inspirations that occurred during the mechanical stimulation trials, both with and without a subsequent expiratory component, and we termed these events inspiratory cough-like responses (ICLRs).

Monitored cardiorespiratory parameters were measured in related periods during 3–10 consecutive breathing cycles. These parameters were taken in cats under the “coughing” protocol just before the last preinjection cough trial (in the control cough period) and right after the codeine microinjection (in the postinjection period). In animals under other protocols, these data were measured just before intraarterial injections (aCSF microinjection), then before the DLH microinjection (during the 1-min waiting period), and at the time of maximum alteration of each parameter (during 0 – 2 min after the DLH application)."
(within ~20 s) injected into the vertebral artery. Approximately 1 min after the beginning of the intra-arterial injection, DLH was microinjected again. We repeated this sequence up to at least five intra-arterial saline applications followed by DLH microinjections in the control group of animals. The treatment group received codeine beginning at the third trial in the sequence in increasing cumulative doses of 0.01, 0.03, and 0.1 mg/kg (33, 100, and 330 nmol/kg) followed by DLH microinjection (within ~1 min). The peaks in the ABD moving average during the responses were all normalized to the first postsaline DLH response. Responses obtained from the second up to the fifth injection (second to fifth saline injections or second saline followed by three codeine injections) were statistically analyzed.

“Codeine-DLH microinjection” protocol. Six animals were used for this protocol, and one of these animals was also used for the cough protocol described above. Three-barrel micropipettes or composite microelectrodes were used to microinject aCSF, codeine (3.3 mM), and DLH (30 mM) into the same medullary location. DLH was microinjected first to confirm the activation of expiratory premotoneurons by increased ABD EMG activity. Ten minutes later, aCSF was microinjected (30–50 nl) followed by DLH (20–40 nl) within 1 min. This sequence (aCSF-DLH microinjections) was repeated after the 10-min waiting period to confirm a stable ABD response. Ten min later, codeine (3.3 mM, 30–40 nl) was microinjected followed by the microinjection of DLH. The peak moving average of ABD EMG induced by DLH was normalized to the DLH response induced by the first (pre-aCSF) DLH microinjection. The first two aCSF-DLH microinjections with the next codeine-DLH microinjection were compared statistically.

After the experiment, the caudal medulla was removed for histological processing. The tissue was fixed in 4% paraformaldehyde followed by a 30% sucrose solution. The frozen medulla was then cut into transverse slices (thickness: 50 or 100 μm) by a freezing microtome. Sections were examined under light and UV microscopy for the detection and localization of injection sites. We identified 14 of 15 codeine microinjection spots under the coughing protocol (Fig. 1). In five of these locations, the composite micropipette was used, and multunit expiratory activity was recorded. In the control group of cats with aCSF microinjections, we identified 9 of 10 locations (Fig. 1). The composite micropipette was used once in these control animals, and expiratory multunit activity was identified. The main precondition for initiating the protocols with DLH microinjections was a stable ABD response induced by DLH (18 of 19 DLH microinjection sites were identified).

Results are expressed as means ± SE. For statistical analysis, repeated-measures ANOVA with Student-Newman-Keuls post tests or, when the dataset did not pass the normality test, the Friedman test with Dunn post test was applied. An unpaired t-test was used in statistical comparisons of data from codeine- versus aCSF-microinjected animals. Cardiorespiratory parameters before and after the codeine microinjection under the coughing protocol were analyzed using a paired t-test. Differences of variables were considered significant if P < 0.05.

RESULTS

Codeine microinjections in the area of the cVRC (28.4 ± 0.7 nl, range: 20–32 nl, 16 injections, 15 locations, 9 cats; Fig. 1) reduced tracheobronchial cough (Fig. 2). The CN (by 29%), expiratory EP amplitudes (by 33%), and the amplitudes of expiratory-related cough ABD EMG activity (ipsilateral by 35% and contralateral by 49%) were significantly reduced within 0–5 min after the codeine microinjections compared with the control (Fig. 2 and Table 1) as well compared with aCSF microinjection values. There was no significant effect of codeine microinjection on inspiratory EP and PS EMG amplitudes during cough (Table 1), although the PS activity immediately after the codeine administration tended to decrease (Fig. 2 and Table 1). Analysis of ICLR (Fig. 2 and Table 1) showed a nonsignificant alteration in the number of responses but a significant reduction in the amplitudes of the contralateral PS EMG moving average (by 22%; Table 1). However, no significant differences were found when codeine microinjection ICLR data were compared with aCSF microinjection ICLR data.

The suppression of cough lasted ~10 min after the microinjection (Table 1). In the period 10–15 min postcodeine microinjection, the CN and expiratory parameters of coughing (Table 1) tended to return back to control values. Inspiratory cough (as well as ICLR) parameters were larger than those in the precodeine control period (Table 1). There were no significant differences in the amplitudes of laryngeal PCA or ThAr EMG moving averages in any of the four laryngeal phases of cough reflex (53) in the postcodeine cough trials compared with the control.

We found no significant effect of codeine microinjection on the total cough cycle duration (CT_total), CT_i, or CT_e (Table 1; as for the phase duration of ICLR), even when CT_i or CT_e...
were expressed as percentages of the control CT_total. However, there was a moderate but significant shortening of the cough ABD EMG duration after the microinjection of codeine (by 22%). The shorter ABDS activation during cough did not return to control levels in the period 10–15 min postcodeine microinjection, even though other cough parameters returned close to control levels in the period 10–15 min postcodeine microinjection. Similarly, we found no significant differences in cardiorespiratory parameters induced by microinjection of DLH except the already described effect of a high dose of codeine intra-arterially on the ABD response.

**DISCUSSION**

The major finding of this study is that the administration of codeine by microinjection into the cVRC suppressed the cough reflex. We also observed reduced ABD excitation due to DLH microinjection in the cVRC when codeine was administered to the brain stem circulation. However, when codeine was microinjected into the cVRC, it did not attenuate the increased ABD discharge caused by microinjection of DLH.

The microinjections (codeine and DLH) made in these experiments were in very near to the nucleus retroambiguus (Fig. 1), a region that is associated with a high concentration of expiratory premotor neurons (4, 27). The DLH microinjections excited these neurons because we observed increases in ABD motor discharge (12, 54), cardiac output, and mean arterial pressure (51, 63). In a separate experiment, we administered codeine by microinjection into the same location. ABD responses due to microinjections of aCSF (36.5 ± 2.0 nl) in the same locations.

There were no significant alterations in monitored cardiorespiratory parameters induced either by the intra-arterial application of saline and codeine or by microinjection of aCSF or codeine in our animals under the protocols with DLH microinjections. Similarly, we found no significant differences in cardiorespiratory parameters induced by microinjection of DLH except the already described effect of a high dose of codeine intra-arterially on the ABD response.

**Table 1. Parameters of control, postcodeine, and recovery period coughs**

<table>
<thead>
<tr>
<th></th>
<th>Control (Precodeine)</th>
<th>Postcodeine Period</th>
<th>Recovery (10- to 15-min Postcodeine) Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough number</td>
<td>4.15 ± 0.80</td>
<td>2.94 ± 0.77b</td>
<td>3.68 ± 0.81b</td>
</tr>
<tr>
<td>Cough-related EP, cmH2O</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inspiratory</td>
<td>3.8 ± 0.4</td>
<td>3.8 ± 0.5</td>
<td>4.4 ± 0.5c</td>
</tr>
<tr>
<td>Expiratory</td>
<td>5.5 ± 1.3</td>
<td>3.7 ± 0.9</td>
<td>4.8 ± 0.8</td>
</tr>
<tr>
<td>Cough-related PS, %</td>
<td>9.10 ± 8.7</td>
<td>74.8 ± 7.8</td>
<td>123.8 ± 15.1b</td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>90.4 ± 8.3</td>
<td>74.6 ± 8.1</td>
<td>112.6 ± 12.3a</td>
</tr>
<tr>
<td>Contralateral</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cough-related ABD, %</td>
<td>80.4 ± 8.9</td>
<td>52.0 ± 8.4</td>
<td>72.6 ± 10.1c</td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>81.4 ± 12.8</td>
<td>41.9 ± 10.1b</td>
<td>63.6 ± 12.4</td>
</tr>
<tr>
<td>Contralateral</td>
<td>1.46 ± 0.23</td>
<td>1.62 ± 0.29</td>
<td>1.52 ± 0.30</td>
</tr>
<tr>
<td>CT_total, s</td>
<td>2.06 ± 0.48</td>
<td>1.97 ± 0.35</td>
<td>1.79 ± 0.34</td>
</tr>
<tr>
<td>Duration of ABD, s</td>
<td>3.52 ± 0.58</td>
<td>3.59 ± 0.50</td>
<td>3.31 ± 0.54</td>
</tr>
<tr>
<td>Number of ICLRs</td>
<td>5.20 ± 0.81</td>
<td>4.33 ± 0.82</td>
<td>5.08 ± 0.97</td>
</tr>
<tr>
<td>ICLR EP, inspiratory, cmH2O</td>
<td>3.5 ± 0.4</td>
<td>3.4 ± 0.4</td>
<td>4.0 ± 0.5c</td>
</tr>
<tr>
<td>ICLR PS, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>86.9 ± 9.4</td>
<td>66.9 ± 9.2</td>
<td>112.6 ± 16.5d</td>
</tr>
<tr>
<td>Contralateral</td>
<td>85.9 ± 9.4</td>
<td>67.1 ± 8.5</td>
<td>103.0 ± 13.9d</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 9 cats. EP, expiratory amplitudes of esophageal pressure; PS and ABD, amplitudes of parasternal (PS) and abdominal (ABD) muscle electromyograph moving averages, respectively; CTi and CT_e, inspiratory and expiratory phase duration, respectively; CT_total, total cough cycle duration; ICLR, inspiratory coughlike responses (deep inspirations). *P < 0.05 and †P < 0.01 vs. control values; ‡P < 0.05, §P < 0.01, and ¶P < 0.001 vs. postcodeine values.

Table 2. Cardiorespiratory parameters of codeine-microinjected animals

<table>
<thead>
<tr>
<th></th>
<th>Precodeine</th>
<th>Postcodeine</th>
</tr>
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<tbody>
<tr>
<td>Respiratory rate, breaths/min</td>
<td>18.0 ± 1.8</td>
<td>17.8 ± 2.0</td>
</tr>
<tr>
<td>Expiratory esophageal pressure, cmH2O</td>
<td>1.1 ± 0.4</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>Esophageal pressure amplitude, cmH2O</td>
<td>2.4 ± 0.4</td>
<td>2.3 ± 0.5</td>
</tr>
<tr>
<td>Mean blood pressure, mmHg</td>
<td>123.4 ± 5.8</td>
<td>124.2 ± 5.2</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>190.4 ± 8.0</td>
<td>189.3 ± 8.0</td>
</tr>
<tr>
<td>End-tidal CO2, mmHg</td>
<td>38.2 ± 1.3</td>
<td>38.9 ± 1.6</td>
</tr>
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</table>

Values are means ± SE.
micropipette tip. Interestingly, higher codeine concentrations (16.5 mM) and doses (up to 0.7 nmol) microinjected in preliminary experiments did not elicit effects that were of larger magnitude than the lower doses that we used. The volumes of DLH that were microinjected varied up to 10% in 7 of 83 microinjections. However, this variation in volumes of DLH that were microinjected cannot account for our findings because we confirmed both in the present as well as a previous (e.g., Ref. 54) series of experiments that alterations in the ABD response (e.g., peak ABD moving average) due to slight differences in microinjected volumes (up to 3 nl) were not significantly different.

Codeine is a μ-opioid receptor agonist. However, the antitussive effects of different μ-opioid receptor agonists are variable (20). Codeine has a low binding affinity to opioid receptors (52), remarkably different antitussive versus analgesic efficacy (14, 20, 56), and much lower antitussive than analgesic stereospecificity in the cat (15). Moreover, naloxone, a nonspecific opioid receptor antagonist, only partially blocks the antitussive effect of codeine in the cat (15–17, 49). Several mechanisms that do not involve μ-opioid receptor activation have been proposed to be involved in the antitussive activity of codeine, e.g., interactions of different opioid receptor sub-systems (16), influence of opioids on the neurotransmission of acetylcholine (52), effect of opioids on N-methyl-D-aspartate receptor channels (49, 64), and an involvement of serotonin receptors, Ca²⁺ channels (32–34, 49), and GABAergic mechanisms (49).

Codeine acts within the brain stem (17) to reduce the number and expiratory amplitudes of cough (5–7). This pattern of cough suppression is consistent with a proposed central cough gating mechanism (9) and was observed in our codeine microinjection experiments (reduced CN and both expiratory EP and expiratory ABD EMG amplitudes). However, the number of ICLRs, which represents the number of coughs and additional deep inspirations during the tracheobronchial stimulation (7), was not significantly reduced. We assume that the reduction in CN induced by microinjections of codeine in the cVRC is at least in part due to a depressed expiratory cough component (no active expiration, no cough by definition), resulting in a lower CN but not the number of ICLRs. The contribution of reduced cough expulsions to decreased CN in previous studies is unknown because the number of induced (coughlike) responses based on enhanced inspiratory activities (ICLRs) was not determined. Codeine had a stronger effect on the contralateral side, as shown by a more pronounced reduction and slower recovery of contralateral expiratory ABD amplitudes during cough (Table 1 and Fig. 2). The axons of expiratory premotoneurons cross the midline, descend to the spinal cord, and drive primarily motoneurons on this contralateral side (4, 27). Codeine administered into the brain stem by microiontophoresis reduced the activity of expiratory units (46). However, the control of spontaneous respiratory activity of these neurons may differ substantially from that during cough (8). Engelhorn and Weller (21) showed markedly reduced expiratory neuronal discharge within the cVRC during coughing due to codeine after intravenous administration. This finding, however, does not demonstrate that codeine acts during cough directly on expiratory premotoneurons. Moreover, the authors reported an additional population of nonexpiratory-related neurons in the area of the caudal ambiguous nucleus (21) that was sensitive to codeine. Our codeine microinjections in the identical location of the cVRC where the DLH was microinjected did not alter...
ABD motor activity that was evoked by DLH. This finding suggests that the expiratory motor output was not generally suppressed by codeine. The markedly lower sensitivity of laryngeal cough, expiration reflex, and sneezing to codeine (38) also supports the view that expiratory premotoneurons in the cVRC are not substantially involved in the cough reduction due to our codeine microinjections. All of the aforementioned reflexes share a common expiratory motor output via expiratory premotoneurons of the cVRC (4, 27). A general inhibition of these neurons by codeine would result in the suppression of the expiratory motor component during multiple behaviors at comparable doses. In addition, our single codeine microinjections presumably affected only a limited number of expiratory premotoneurons. These neurons are found in longitudinal and bilateral columns associated with the nucleus retroambiguus (4). Inhibition of a subset of these expiratory premotor neurons by codeine should not lead to the widespread effects that we observed. We have proposed, in accordance with Bongianni et al. (13), that codeine microinjected in the cVRC region may act on a different population of neurons to suppress coughing than expiratory premotoneurons. We (54) recently suggested that DLH-induced excitation of neurons in the cVRC led to the suppression of cough in a manner similar to antitussives. There was a noticeable cough-related neuronal activation within the cVRC as detected by the Fos-immunoreactive method (24, 30). This activation of Fos-positive neurons in the cVRC was reduced by codeine in parallel with the suppression of coughing (24). The presence of recruited neurons during coughing has been reported in the cVRC (21, 28). These neurons may be part of a complex network that produces cough suppression in response to a variety of local neuropharmacological challenges such as glutamate agonists (54), antagonists (13), or codeine in the cVRC (21).

The DLH-induced ABD discharge was reduced by a cumulative dose of codeine applied intra-arterially that was higher than that necessary to suppress cough in other studies (6, 36). Because codeine microinjections within the cVRC had no effect on DLH-induced ABD discharge, we suggest that the suppression of DLH-related ABD activation was due to an action of codeine outside the region of the cVRC. However, we did not attempt to microinject codeine outside the area of the cVRC. There may be multiple areas with neuronal populations sensitive to codeine and other central antitussives involved in the control of coughing and, particularly, expiratory motor output during cough. Kito et al. (37) reported cough suppression by codeine in the dorsal lateral tegmental field, and Oh et al. (50) and Mutolo et al. (45) reported antitussive effects of these drugs within the NTS. In addition, cVRC neurons receive inputs from the NTS, rostral ventrolateral medulla, and medullary reticular formation of the FTL, raphe, and dorsolateral pons (4, 27, 41, 60), all areas that contain neurons involved in an expression of cough (24, 30).

Cough I amplitudes (PS EMGs or EP) were not significantly reduced in our experiments. However, the reduction of inspiratory PS EMG amplitudes during ICLR was statistically significant on the contralateral side from the microinjection (Table 1), suggesting that codeine within the cVRC had a suppressive effect on cough inspiratory activity. Codeine injected into the peripheral or brain stem circulation had no effect on cough inspiratory motor output and significantly decreased only CN and expiratory motor output (5–7). On the other hand, codeine has been shown to have a significant effect on power spectra and other discharge parameters of phrenic nerve activity during cough in anesthetized dogs (26). Blockade of glutamate synaptic transmission within the cVRC of the rabbit eliminated both the inspiratory as well as expiratory component of cough (13). The authors proposed the existence of a neuronal population within the cVRC that was involved in expression of tracheobronchial cough. It is very unlikely that expiratory premotoneurons are involved in the reduction of inspiratory motor output during cough (Refs. 13 and 54; see also our previous discussion on cough expiratory motor output). The spontaneously active expiratory premotor units have very few axon collaterals at the level of the brain stem (22, 41), although there are generally rich axonal projections from the cVRC to the rest of the respiratory network (60). The effects of codeine microinjections in the cVRC on inspiratory and expiratory motor output during cough support the concept of different regulation of inspiratory and expiratory components of the cough motor pattern (9). Similarly, our laryngeal cough data are consistent with the differential regulation of cranial (e.g., laryngeal) and spinal motor outputs (2, 25, 53, 54, 59). Depression of laryngeal motor output by central antitussives has been reported (44), but the doses of drugs that were used were almost an order of magnitude higher than those required to produce an antitussive effect (62).

The only altered (shortened) temporal cough parameter in our experiment with microinjection of codeine into the cVRC was the duration of cough ABD activity. Unfortunately, there is no information about the changes in the duration of cough ABD activation under the effects of codeine or other central antitussives (7, 9) administered intravenously or intra-arterially.

The recovery process was variable in duration in different animals, presumably depending on the sensitivity of a particular animal to codeine, on the depth of effects caused by codeine, and on the rate of the drug elimination. The magnitude of recovery at the 10- to 15-min time point was not uniform across all measured parameters. This uneven recovery process has been observed before, e.g., in our study (54) with DLH microinjections.

Codeine administered via the intravenous or intra-arterial routes (1, 7, 14, 65) in doses that strongly reduced coughing induced low or no alterations in respiratory and cardiovascular parameters. We saw very little alterations in cardiorespiratory parameters caused by microinjections of codeine in the cVRC. This area is not critically involved in the generation of breathing (4, 27, 29).

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DISCLOSURES

No conflicts of interest are declared by the author(s).

REFERENCES


CODEINE AND COUGH


