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Role of the dorsomedial medulla in suppression of cough by codeine in cats

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ABSTRACT

The modulation of cough by microinjections of codeine in 3 medullary regions, the solitary tract nucleus rostral to the obex (rNTS), caudal to the obex (cNTS) and the lateral tegmental field (FTL) was studied. Experiments were performed on 27 anesthetized spontaneously breathing cats. Electromyograms (EMG) were recorded from the sternal diaphragm and expiratory muscles (transversus abdominis and/or obliquus externus; ABD). Repetitive coughing was elicited by mechanical stimulation of the intrathoracic airways. Bilateral microinjections of codeine (3.3 or 33 mM, 54 ± 16 nl per injection) in the cNTS had no effect on cough, while those in the rNTS and in the FTL reduced coughing. Bilateral microinjections into the rNTS (3.3 mM codeine, 34 ± 1 nl per injection) reduced the number of cough responses by 24% ($P < 0.05$), amplitudes of diaphragm EMG by 19% ($P < 0.01$), of ABD EMG by 49% ($P < 0.001$) and of expiratory esophageal pressure by 56% ($P < 0.001$). Bilateral microinjections into the FTL (33 mM codeine, 33 ± 3 nl per injection) induced reductions in cough expiratory as well as inspiratory EMG amplitudes (ABD by 60% and diaphragm by 34%; $P < 0.01$) and esophageal pressure amplitudes (expiratory by 55% and inspiratory by 26%; $P < 0.001$ and 0.01, respectively). Microinjections of vehicle did not significantly alter coughing. Breathing was not affected by microinjections of codeine. These results suggest that: 1) codeine acts within the rNTS and the FTL to reduce cough in the cat, 2) the neuronal circuits in these target areas have unequal sensitivity to codeine and/or they have differential effects on spatiotemporal control of cough, 3) the cNTS has a limited role in the cough suppression induced by codeine in cats.

1. Introduction

There have been significant advances in our understanding of pathological cough, its symptoms, classification, diagnostic procedures and treatment (Morice et al., 2007; Dicpinigaitis et al., 2009; Plevkova and Song, 2013). Some progress has been made in our knowledge of cough neuronal circuits (Haji et al., 2013; Pitts et al., 2016) from the introduction of the revolutionary computational model of the cough generating neuronal network (Shannon et al., 1998) and the first complex description of cough brainstem neuronal circuitry in cat (Oku et al., 1994; Gestreau et al., 1997; Shannon et al., 2004; Jakus et al., 2008). Further significant knowledge has been gained during last decade about cough-related neurotransmission and neuromodulation at the central level (Bolser, 2009; Cinelli et al., 2015) including the mechanisms and sites of action of antitussive drugs (Mutolo et al., 2010; Poliacek et al., 2010). However, significant gaps remain in our understanding of the central regulatory mechanisms for coughing. Therefore, the treatment of excessive or insufficient cough is mostly empirical and not based on specific knowledge of neuronal mechanisms responsible for alterations in the cough reflex.

Codeine is very effective to reduce the number and expiratory efforts of mechanically induced cough from the tracheobronchial tree in cat by an action on the cough central neuronal circuitry within the brainstem (Bolser et al., 1995; Bolser, 2006). Among brainstem areas with significant neuronal components of cough control, central antitussives act within the nucleus of the solitary tract (NTS; Ohi et al., 2005; Mutolo et al., 2008) and particularly codeine works in the reticular formation of the lateral tegmental field (FTL; Kito et al., 1977) and in the caudal ventral respiratory column (Poliacek et al., 2010). The caudal ventral respiratory column contains a high concentration of expiratory premotor neurons. However, this population of expiratory pre-motoneurons, transmitting expiratory motor drive from the brainstem circuits to the spinal motoneurons (Iscoe, 1998), is relatively insensitive to codeine and unlikely to be responsible for cough suppression (Poliacek et al., 2010). In response to challenge of the medullary raphé with codeine, the excitability and motor output of

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tracheobronchial cough was depressed by a small amount (Poliacek et al., 2012). Considering that codeine is one of the most widely prescribed drugs to treat cough, there is still a poor understanding of its mechanisms (Haji et al., 2012, 2013). In fact, the antitussive efficacy of codeine in humans with airway pathology has been questioned (Freestone and Eccles, 1997; Smith et al., 2006; Bolser and Davenport, 2007).

The NTS, where codeine has been proposed to act as an antitussive (Kito et al., 1977; Ohi et al., 2005), is a primary projection site of vagal afferents, including cough-related afferents, and the location of 2nd order interneurons of the cough reflex arc (Kalia and Mesulam, 1980; Kubin et al., 2006). However, older microinjection experiments (Kito et al., 1977; Ohi et al., 2005) employed doses of codeine (10 and 1 μg) that were large and not restricted to the immediate region of the injection site (Nicholson et al., 2000). Therefore, the surrounding regions of the reticular formation e.g. the FTL, that contain important cough control elements (Jakus et al., 2000, 2008; Haji et al., 2012), may include cough-related neuronal populations that are sensitive to codeine and could have contributed to the antitussive effects that have been observed by others (Kito et al., 1977). In addition, we re-examined the NTS for its role in the responsiveness, motor drive and temporal features of cough using kynurenic acid and showed very different contributions of the NTS regions caudal *vs.* rostral to the obex in cat (Poliacek et al., 2017).

We hypothesized that codeine administered locally in the NTS caudal to the obex (cNTS), in the NTS rostral to the obex (rNTS) and in the FTL would have suppressive effect on cough and the extent of this suppression would differ between these target areas. We also hypothesized that codeine microinjections in these brainstem locations would elicit a specific mode of action that is similar to that observed after systemic administration i.e. no changes in the cough temporal features and cough inspiratory motor drive.

2. Materials and methods

The experiments were performed on 27 cats (3.77 ± 0.20 kg; 22 males and 5 females) under pentobarbital sodium anesthesia (Morbital Polfa or Pfannenschmidt GmbH; 38 mg/kg iv initially). Supplementary doses were administered (1–3 mg/kg, i.v.) as needed (depending on the presence of reflex withdrawal of the hind limb, corneal and palpebral reflexes, jaw tone, respiratory rate and blood pressure). Atropine (HBM Pharma s.r.o., Martin, Slovakia; 0.1 mg/kg, iv) and hydrocortisone (Valeant Czech Pharma s.r.o., Praha, Czech republic; 2 mg/kg iv) were given to reduce secretions and brain swelling, respectively. The trachea, femoral artery and vein were cannulated. A balloon catheter was inserted into the esophagus for the measurement of esophageal pressure (EP). All animals were allowed to spontaneously breathe an oxygen-enriched air (30–40%). Body temperature was maintained at 38.0 ± 0.5 °C by a heating pad and continuously monitored together with blood pressure, EP, respiratory rate and end-tidal CO_2 concentration ($ETCO_2$). Samples of arterial blood were periodically removed to monitor blood gases and pH to maintain these values within appropriate ranges. Animal care as well as all procedures were performed in accordance with the Animal Welfare Guidelines of the Comenius University and the legislation of animal use and welfare of Slovak Republic and European Union (the Directive 2010/63/EU of the European Parliament) as well as U.S.A.

Electromyograms (EMGs) were recorded from expiratory abdominal muscles (transversus abdominis and/or obliquus externus; ABD), and an inspiratory muscle (sternal diaphragm; DIA), with bipolar teflon insulated fine steel wire hook electrodes (peeled off 3 mm of electrode tip). Tracheobronchial cough was elicited by mechanical stimulation of the intrathoracic airways with a soft polyethylene catheter. The stimulation catheter was moved periodically back and forth in the trachea 6–8 times for periods of 10 s to elicit repetitive coughing (the same experimenter conducted all stimulations).

Animals were placed prone in a stereotaxic frame and the dorsal surface of medulla was exposed. The surface of the brainstem was covered by warm paraffin oil. Microinjections of codeine (3.3 mM or 33 mM; Dr.Kulich Pharma s.r.o. Hradec Králové, Czech Republic) were performed in the NTS or FTL. The drug was dissolved in artificial cerebrospinal fluid that was prepared in our laboratory (aCSF; ALZET Osmotic Pumps, California, USA; Poliacek et al., 2012). Single barrel glass micropipettes (tip diameter 15–60 μm) were used for pressure microinjection of solutions. We did not employ neuronal recordings as a guidance for our microinjections because a presence of similar neurons in all target areas (King and Knox, 1984; Haji et al., 2012) and large difficulty of combining recording and microinjection procedure when bilateral delivery of codeine that has to be accomplished quickly (Poliacek et al., 2010). The micropipette was driven ventraly from the dorsal approach under stereotaxic control until the tip was positioned: in the cNTS (1.0–1.2 mm caudal to the obex, 1.1-1.2 mm lateral to the midline, 1.3–1.5 mm below the dorsal medullary surface), in the rNTS (1.2 mm rostral to the obex – for aCSF microinjections at 0.7 and 1.6 mm rostral to the obex, 2.4–2.5 mm lateral to the midline, 1.7–1.8 mm below the dorsal medullary surface) and in the region of FTL (2.1–2.4 mm rostral to the obex, 2.3–2.5 mm lateral to the midline, 2.2–2.5 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 (the shift in the position of meniscus determines the volume of cylinder ejected from the pipette). Average microinjected volumes are reported for each target area in the results section. Injection sites were labelled by fluorescent latex beads (Eugene, Oregon, U.S.A.) that were suspended in the injectate (Poliacek et al., 2007). The positions of the micropipette tips were confirmed by the presence of a labelled spot in or near the required medullary area (commissural sub-nucleus of the NTS for the cNTS, ventrolateral subnucleus of the NTS and/or close proximity of the tractus solitarius for the rNTS and reticular formation approximately in the middle between the NTS and the nucleus ambiguus for the FTL (Fig. 1)).

Cough was defined by a large burst of inspiratory related DIA EMG activity, immediately followed by a burst of expiratory ABD EMG activity, and by a related negative to positive EP change. These criteria separate cough from other defensive airway behaviors such as expiration reflex (Poliacek et al., 2008) or swallow (Pitts et al., 2013).

All EMGs were amplified, filtered (100–3000 Hz) and recorded, then rectified and integrated (moving average with the time constant 200 ms). The number of coughs (average number of coughs per 10 s stimulation) in response to mechanical stimulation of the tracheobronchial airway (cough number = CN) was recorded. For each cough, the maximum amplitude of DIA and ABD EMG moving averages and inspiratory and expiratory amplitudes of EP during the appropriate phase of cough were analyzed. The durations of: cough related DIA and ABD activation (TDIA, TABD); the augmenting segment of cough DIA activity (CTI); the active cough expiratory period (from the peak of DIA activity to the end of cough related ABD bursting; CTE1); the passive quiescent period of cough expiration (from the end of the cough related ABD activity to the beginning of the next cough/respiratory cycle; CTE2); the total expiratory duration (CTE = CTE1 + CTE2; from the peak of cough DIA activity to the next cycle); the overlapping of DIA and ABD burst (Over); the time between maxima of DIA and ABD activity (Dif); the duration of all cough related EMG activity (Tactive = CTI + CTE1); and the total cough cycle duration (CTtot) were analyzed (Poliacek et al., 2017). The maxima were determined using moving average signals, the onsets and offsets of muscle activation using raw EMG signals.

Monitored cardio-respiratory parameters were measured during 3 consecutive breathing cycles before the first microinjection (for the control pre-microinjection conditions) and approximately 1 min after the last microinjection (for the post-microinjection conditions) before the first stimulus occurred in order to minimize the effects of airway stimulation on these data.