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Central Mechanisms II: Pharmacology of Brainstem Pathways

D.C. Bolser

Department of Physiological Sciences, University of Florida, College of Veterinary Medicine, Gainesville, FL 32610-0144, USA

D.C. Bolser: bolser@ufl.edu

Abstract

Following systemic administration, centrally acting antitussive drugs are generally assumed to act in the brainstem to inhibit cough. However, recent work in humans has raised the possibility of suprapontine sites of action for cough suppressants. For drugs that may act in the brainstem, the specific locations, types of neurones affected, and receptor specificities of the compounds represent important issues regarding their cough-suppressant actions. Two medullary areas that have received the most attention regarding the actions of antitussive drugs are the nucleus of the tractus solitarius (NTS) and the caudal ventrolateral respiratory column. Studies that have implicated these two medullary areas have employed both microinjection and in vitro recording methods to control the location of action of the antitussive drugs. Other brainstem regions contain neurones that participate in the production of cough and could represent potential sites of action of antitussive drugs. These regions include the raphe nuclei, pontine nuclei, and rostral ventrolateral medulla. Specific receptor subtypes have been associated with the suppression of cough at central sites, including 5-HT_{1A}, opioid (μ , κ , and δ), GABA-B, tachykinin neurokinin-1 (NK-1) and neurokinin-2, non-opioid (NOP-1), cannabinoid, dopaminergic, and sigma receptors. Aside from tachykinin NK-1 receptors in the NTS, relatively little is known regarding the receptor specificity of putative antitussive drugs in particular brainstem regions. Our understanding of the mechanisms of action of antitussive drugs would be significantly advanced by further work in this area.

1 Introduction

The pharmacology of centrally active antitussive drugs is a multifactorial topic that involves not only pharmacological and pharmacokinetic issues but neurophysiology as well. This review will focus on three primary matters related to the brainstem actions of these drugs: (1) location of action, (2) identity of neurones affected by the drugs, (3) receptor specificity. There are other informative reviews available (Reynolds et al. 2004).

2 Location of Action of Antitussive Drugs

It is widely accepted that several prominent drugs act in the central nervous system to inhibit cough, primarily by an action in the brainstem. The evidence supporting this concept is strong and is based largely on studies showing that decerebrate animals can cough and that antitussives will suppress coughing under these circumstances (Chou 1975; Wang et al. 1977; Domino et al. 1985; Lal et al. 1986; Bolser 1991; Bolser and DeGennaro 1994; Gestreau et al. 1997; Ohi et al. 2004, 2005).

The central control of cough is complex and there may be many potential sites in the brainstem at which a given drug may act to suppress this behavior. In this context, an understanding of the brainstem regions that may be involved in the production of cough is an important component of any approach to the investigation of the actions of antitussive drugs. It is critical to know “where to look” to design studies investigating the mechanisms of action of these

agents. In this control system there may be many areas where antitussives could work, but only a few that are responsible for the cough-suppressant effects that result from systemic administration of these agents. It should be noted that the results of studies showing a brainstem action of antitussives do not preclude an effect of these drugs on suprapontine or spinal pathways in animals that have an intact neuraxis.

Spinal motoneurons (and their antecedant interneuronal pathways) are an often-overlooked component of the cough-generation system, but represent an important site at which regulation of the behavior can occur. Several classes of compounds that have antitussive activity also suppress spinal motor activity in other systems. Baclofen is a well-known muscle relaxant and inhibits spinal motor activity in low doses after intrathecal administration (Penn 1992). Opioids also inhibit motor activity after topical administration to the spinal cord in spinal cats (Schomburg and Steffens 1995). Central nervous system penetrant drugs gain access to the entire neuraxis within 5 min after vascular administration and compounds that are delivered to the cerebrospinal fluid (CSF) of the brain are rapidly transported to the spinal CSF (Xie and Hammarlund-Udenaes 1998). Therefore, centrally acting antitussive drugs probably reach the spinal cord after systemic administration. Preliminary results (Rose et al. 2004) have shown that intrathecal administration of baclofen has no effect on expiratory muscle electromyographic activity during tracheobronchial cough. However, the same dose of baclofen almost completely inhibits cough when administered via the vertebral artery. Similar results were obtained in preliminary studies with intrathecal administration of codeine. These preliminary findings are consistent with disfacilitation of expiratory spinal motor pathways by antitussive drugs acting in the brainstem.

The role of suprapontine pathways in the generation of cough and the effects of antitussive drugs is not well understood. It is likely that the potential role of these areas in the generation of cough may be much greater in conscious humans (and perhaps animals as well), given that humans can both initiate and suppress cough by voluntary means (Hutchings et al. 1993; Hutchings and Eccles 1994). Significant sensations also are associated with irritant-induced cough, indicating the involvement of suprapontine sensory systems during coughing. A model incorporating the potential influence of suprapontine pathways in the production of cough has recently been published (Bolser 2006). However, codeine has no effect on sensations during irritant-induced cough in humans, but in that study this opioid did not alter the musculomechanical aspects of cough (Davenport et al. 2007). In the absence of an effective antitussive agent in humans, the role of suprapontine pathways and sensations in the mechanism of action of these drugs will remain obscure.

3 Identity of Neurones Affected by Antitussive Drugs

A great deal of information is available regarding the locations of neurones that participate in the production of cough in the brainstem. Shannon and coworkers (Shannon et al. 1996, 1998, 2000; Baekey et al. 2001) have developed a model of the neurogenic mechanism for coughing that includes columns of neurones in both the ventrolateral and the dorsomedial regions of the medulla. Different classes of neurones in these regions interact with one another to control inspiratory- and expiratory-phase durations during cough, the magnitude of motor drive to spinal motoneurons, and the activation of laryngeal muscle motoneurons that determine the caliber of the larynx (Shannon et al. 1996, 1998, 2000; Baekey et al. 2001). These neurones also participate in the control of breathing. Sensory input to this cough pattern generation system is mediated by relay neurones in the nucleus of the tractus solitarius (NTS), located in the dorsomedial medulla.

Recent studies utilizing microinjection of a neurotoxin to induce chemical lesions have implicated the pons (Poliaček et al. 2004), raphe nuclei (Jakus et al. 1998), and lateral tegmental

field (Jakus et al. 2000) in the production of coughing. The microinjection method is best suited to the study of regions or nuclei of the brain that are roughly spherical because the diffusion pattern of microinjected chemicals approximates a sphere (Lipski et al. 1988). Use of this method in the study of the actions of the raphe nuclei presents unique issues because these nuclei are restricted to the midline in a “sheet” extending dorsoventrally. The lesions produced by Jakus and coworkers (Jakus et al. 1998) affected the raphe nuclei but extended into the nearby medial reticular formation as well, potentially implicating this region in the production of cough (Fig. 1).

Xu et al. (1997) have shown that one of the deep cerebellar nuclei, the nucleus interpositus, has an important modulatory role in the production of cough. Furthermore, the inferior olive, a brainstem nucleus that interacts with the deep cerebellar nuclei, was labeled in coughing animals in a *cFos* study (Gestreau et al. 1997).

From a theoretical standpoint, any of these areas could represent targets for the action of antitussive drugs. Important considerations are the route and dose of the cough-suppressant drug in question. Intracerebroventricular or vascular administration of antitussive agents lacks anatomic specificity (Bolser 1996). Vascular administration can be coupled with electrophysiological recordings of single neurones to provide anatomic specificity, but the drug will still reach all the tissue supplied by the brainstem circulation (Jakus et al. 1987). Intravenous or intra-arterial administration of antitussive drugs does have the advantage that the effects can be directly related to concentrations of the agents that are restricted to the therapeutic range. Effective concentrations of cough suppressants administered by the intracerebroventricular route also can be related to therapeutic doses obtained by vascular administration but only by the use of ratios (Bolser 1996). However, a drawback of intracerebroventricular studies is that doses are often not normalized to body weight.

The specific location at which an antitussive drug acts to suppress cough can be studied with the microinjection method. Microinjection has the advantage of localizing the drug to a relatively small region of the brain. However, the drug can still diffuse 500–1,000 μm away from the injection site depending on factors such as volume injected, concentration, and the characteristics of the extracellular space (Lipski et al. 1988). An additional limitation of this method is that it is difficult to relate the dose of a drug that is delivered to therapeutic concentrations associated with systemic administration. Microinjection studies run the risk of delivering doses of cough suppressants that result in tissue concentrations that are higher than those that would be achieved after systemic administration. As such, the risk of type I errors in these types of studies may be high.

3.1 Nucleus of the Tractus Solitarius

Antitussive drugs have been microinjected into the region of the NTS. Codeine and dextromethorphan have been injected into the NTS in relatively large quantities (0.5–10 μg) in large volumes (0.5–10 μL), resulting in suppression of cough in the cat and guinea pig. As stated already it is difficult to know whether these amounts of antitussive drugs are consistent with local tissue concentrations that occur after systemic administration. Concentrations of microinjected drugs are usually restricted to the nanomolar range with volumes of 100 nL or less to limit the local concentration of the drug (Lipski et al. 1988). Gestreau and coworkers (Gestreau et al. 1997) have shown that *cFos* labeling of NTS neurones is reduced after systemic administration of a large dose of codeine (17 mg kg^{-1}), which they suggested occurred because codeine acted on this population of neurones. Recent work suggests that codeine presynaptically inhibits glutaminergic excitatory neurotransmission from primary afferent fibers to putative second-order interneurons in the NTS (Ohi et al. 2007). Furthermore, glycinergic currents in acutely dissociated guinea pig NTS neurones were inhibited by dextromethorphan (Takahama et al. 1997). Similar findings were obtained for NTS neurones

in slices of the guinea pig medulla for the effects of substance P on excitatory postsynaptic potentials induced from electrical stimulation of the tractus solitarius (Sekizawa et al. 2003). In this study, the authors specifically identified second-order interneurons by labeling of primary afferents with retrograde tracer. Another study by this group on NTS neurons from brainstem slices of nonhuman primates showed that substance P in the micromolar range decreased input resistance of these cells, indicative of inhibition (Chen et al. 2003). This effect was observed in control as well as ozone-challenged animals. Interestingly, tachykinin neurokinin-1 (NK-1) receptor antagonists also depressed the responses of these neurons to current injection, but not the excitatory effect of electrical stimulation of the tractus solitarius (Chen et al. 2003). Mutolo et al. (2007) have shown in the rabbit that microinjection of picomolar quantities of substance P into the commissural subnucleus of the NTS results in enhancement of coughing. Another study showed central suppression of cough by tachykinin NK-1 antagonists (Bolser et al. 1997) delivered to the brainstem circulation. The apparent conflicting findings of some of these studies may simply be a manifestation of the inherent complexity of the neural network for cough in this region of the medulla. Antitussive drugs may act presynaptically, postsynaptically, and/or on multiple subpopulations of interneurons in this area, presenting significant challenges to the interpretation of microinjection studies.

3.2 Ventrolateral Respiratory Column

Jakus and coworkers (Jakus et al. 1987) have shown that the tracheobronchial cough-related discharge of caudal ventral respiratory group expiratory neurons is reduced after systemic administration of codeine. However, this observation does not prove that antitussive drugs act directly on any of these neuron groups. The findings of Jakus and coworkers (Jakus et al. 1987) could be explained either by an inhibitory action of codeine on medullary expiratory neurons or by an action of this drug on other neurons that provide excitatory input to medullary expiratory neurons. Jakus et al. (1987) observed that previously silent caudal expiratory neurons were recruited during cough in the cat. These neurons have expiratory discharge patterns during cough that are very similar to abdominal motor discharge patterns. Merrill (1970, 1972) investigated spinal projections of both spontaneously active caudal expiratory neurons and nearby silent neurons. Both groups of neurons had contralateral spinal axons and Merrill (1974) noted that antidromic latencies of the silent neurons fluctuated with the respiratory cycle, indicating that these neurons had respiratory-modulated membrane potentials. Indeed, he suggested that this group of silent neurons was recruited during expulsive movements, such as cough.

Microinjection of the excitatory amino acid agonist D, L-homocysteic acid (DLH) into the caudal portion of the ventral respiratory group elicited prolonged suppression of coughing (more than 10min) but only brief (less than 1min) alterations in breathing in the cat (Poliacek et al. 2007). Spontaneously active neurons in this region of the medulla are almost exclusively premotor expiratory with few, if any, axon collaterals to the rest of the brainstem. These neurons are unlikely to have mediated the cough-suppressant effects that were observed. Given that DLH is an excitatory neurochemical, we have proposed that either silent or nonbreathing modulated neurons in or near the caudal ventral respiratory group are responsible for this cough suppression. We also have preliminary data showing that microinjection of codeine into this area in doses as low as 100 pmol suppresses cough. Given that codeine and the well-known excitatory neurochemical DLH have similar effects, it is possible that codeine also is acting as an excitatory agent under these conditions. This proposal represents a departure from the long-held concept that centrally acting antitussives *inhibit* the activity of neural elements involved in the production of cough. An alternative hypothesis is that these drugs may excite neurons in the central nervous system that in turn synaptically inhibit control elements in the cough network.

4 Receptor Specificity

The presence of receptors for a drug in a given brain region does not indicate that the drug will exert its effects by an action in that location. The receptor density and/or affinity for the drug may be low in that region, requiring a dose of the drug to alter the behavior of these neurones that is out of the therapeutic range. Furthermore, many of the brain regions that contain neurones associated with the production of cough also mediate other functions, such as breathing and cardiovascular control. The concept of receptor specificity is relevant not only to the receptor subtype actuated by a given drug, but also to the physiological system that is affected. For example, both morphine (a specific μ -opioid receptor agonist) and baclofen (a specific GABA-B receptor agonist) are known to be respiratory depressant agents (Adcock et al. 1988; Hey et al. 1995). However, in the guinea pig the doses of these drugs necessary for respiratory depression are greater than those required for inhibition of cough (Adcock et al. 1988; Bolser et al. 1994; Hey et al. 1995). Presumably, each drug acts specifically at its respective receptor subtype to inhibit both cough and breathing, but the potency of the drugs to inhibit these behaviors is different.

Studies to identify the receptor specificity of antitussive drugs have been restricted to animal models. In humans, investigations of the effects of antitussive drugs are usually focused on demonstrating efficacy. Studies in animal models usually involve the use of specific agonists and/or antagonists to demonstrate receptor specificity.

Opioids, most notably codeine, are effective cough suppressants in animal models (May and Widdicombe 1954; Chou 1975; Wang et al. 1977; Adcock et al. 1988; Bolser et al. 1993, 1999; Bolser and DeGennaro 1994; Reynolds et al. 2004), although the efficacy of codeine in humans has recently been questioned (Freestone and Eccles 1997; Smith et al. 2006). In guinea pigs, the action of codeine as a cough suppressant has been attributed to μ -opioid receptors (Kotzer et al. 2000). However, codeine does not appear to act through opioid receptors in the cat (Chau et al. 1983), because its antitussive action is not blocked by the opioid antagonist naloxone in this species. The particular receptor that codeine acts through to inhibit cough in the cat is unknown. The μ -opioid receptor agonist morphine does inhibit cough in the guinea pig and cat by a naloxone-sensitive (or naltrexone-sensitive) mechanism (Chau et al. 1983; Adcock et al. 1988). Morphine has been shown to be active as an antitussive in humans (Fuller et al. 1988; Morice et al. 2007). Furthermore, both δ - and κ -opioid receptor agonists inhibit cough in the guinea pig. Opioid antagonists do not influence the cough reflex in either animals or humans (Chau et al. 1983; Freestone and Eccles 1997; Kotzer et al. 2000), suggesting that endogenous opioids are not critical for the production of cough. These observations are consistent with the existence of a regulatory mechanism of endogenous opioids on cough that is normally quiescent. The conditions under which this putative mechanism could be active are unknown. Moreover, the role(s) that the different opioid receptor subtypes may have in the regulation of the expression of coughing in the human is not clear.

GABA-B receptor agonists can inhibit cough in animals and humans (Bolser et al. 1993, 1994, 1999; Dicipinigitis 1996; Dicipinigitis and Dobkin 1997; Dicipinigitis et al. 1998). These effects are central in both guinea pigs and cats and the efficacy of baclofen, the prototypical centrally active GABA-B receptor agonist, is similar to that of opioids in animal models (Bolser et al. 1993). A specific receptor antagonist for GABA-B receptors, SCH 50911, had no effect on its own to alter cough but did block the antitussive effects of baclofen in the cat and guinea pig (Bolser et al. 1995). This observation suggests that γ -aminobutyric acid (GABA), acting through the GABA-B receptor subtype, does not modulate the production of cough under experimental conditions. The actions of other GABA receptor subtypes on the expression of cough are not well understood. The GABA-A receptor antagonist bicuculline induces seizures (Wood 1975). This limits the usefulness of bicuculline in the investigation of

the effects of GABA-A receptors on cough as seizure activity itself may modify the excitability of coughing. The effects of GABA-C receptors on cough are unknown.

Serotonin receptor ligands can alter cough in animal models. Most of our current information indicates that 5-HT_{1A} receptor agonists inhibit cough in small animals (Kamei et al. 1991a; Stone et al. 1997). Serotonin may modify the effects of other antitussive drugs, suggesting more complex actions of these drugs than simple suppression of a single type of neurone in the central nervous system. For example, in the rat depletion of serotonin attenuated the antitussive effect of morphine, dihydrocodeine, and dextromethorphan (Kamei et al. 1987b). The serotonin precursor L-tryptophan potentiated the antitussive action of dihydrocodeine (Kamei et al. 1990) and the serotonin antagonist methysergide reduced the antitussive effects of dextromethorphan and dihydrocodeine in rats (Kamei et al. 1986a, 1996). L-Tryptophan treatment also prevented the development of tolerance in rats to the antitussive effects of dihydrocodeine (Kamei et al. 1991b). Morphine dependence elicits a reduced sensitivity to opioid and non-opioid antitussives and these effects were associated with a reduction on serotonin receptors in the brainstems of morphine-dependent rats (Kamei et al. 1989). In cats, the serotonin precursor 5-hydroxytryptophan inhibited cough (Kamei et al. 1986b) and methysergide increased cough number and reduced the antitussive effects of dextromethorphan (Kamei et al. 1986a). In humans, serotonin and 5-hydroxytryptamine both inhibited cough due to inhalation of low-chloride solutions, but not coughing elicited by capsaicin aerosols (Stone et al. 1993). Our understanding of the effects serotonin receptor ligands on cough would be enhanced by further work in animal models other than the rat as well as in the human.

The data from the cat on the effects of serotonin receptor agonists and antagonists on cough appear to be consistent with the data from experiments conducted in rats. However, the rat model has been used by relatively few investigators owing to the comparative difficulty to induce cough in this species. Furthermore, a detailed analysis of the motor patterns induced by mechanical stimulation of the tracheo-bronchial region as well as inhalation of capsaicin in the rat revealed that the behaviors produced by these interventions did not conform to the well-accepted criteria for cough (Ohi et al. 2004). There also is a considerable amount of published information on antitussives and cough in the mouse (Kamei et al. 1993a–f, 1994a–d, 1995a, b, 1999a, b, 2003a, b, 2006a, b, 2007; Morita and Kamei, 2003), but there is no corresponding analysis of the motor patterns of putative coughing in this species to confirm that the behaviors that were measured were coughs. Indeed, in their monograph, Korpas and Tomori (Korpas and Tomori 1979) indicate that mice do not cough in response to mechanical or chemical stimulation of airway afferents, but they do readily express another airway defensive behavior, the expiration reflex, in response to mechanical stimulation of the larynx. The expiration reflex is regulated differently than cough (Korpas and Tomori 1979; Tatar et al. 2008), is insensitive to antitussive drugs (first shown by May and Widdicombe 1954 and later confirmed by Korpas and Tomori 1979), and is generated by a brainstem neural network that is not organized in the same manner as that for coughing (Baekey et al. 2004).

Tachykinin receptor antagonists have been shown to be effective cough suppressants in the cat, dog, and guinea pig (Advenier et al. 1993; Girard et al. 1995; Kudlacz et al. 1996; Bolser et al. 1997; Hay et al. 2002; Chapman et al. 2004; Joad et al. 2004). Both tachykinin NK-1 and tachykinin neurokinin-2 receptor antagonists can inhibit cough by a central mechanism (Bolser et al. 1997; Joad et al. 2004; Mazzone et al. 2005). Although a tachykinin neurokinin-3 receptor antagonist can inhibit cough (Daoui et al. 1998), it is not known whether this effect is by a central site of action. There is evidence that substance P can enhance cough when microinjected into the region of the NTS in the rabbit (Mazzone et al. 2005; Mutolo et al. 2007). However, substance P inhibited excitatory postsynaptic potentials in guinea pig NTS putative relay neurones that were produced by electrical stimulation of the tractus solitarius (Sekizawa et al. 2003). This effect of substance P was presynaptic and blocked by a tachykinin NK-1 receptor

antagonist (Sekizawa et al. 2003). These observations suggest that tachykinin NK-1 receptors are important in regulating the excitability of cough in the region of the NTS. The exact role that tachykinin receptors have in this complex area is not clear at this time. The extent to which tachykinin receptor antagonists act to regulate cough in the NTS and/or other brainstem areas after systemic administration in animal models is unknown. Only one study on the effects of tachykinin receptor antagonists on cough in humans has been published. Fahy et al. (1995) observed no effect of the tachykinin NK-1 receptor antagonist on hypertonic saline-induced cough in humans with mild asthma. Given the limited amount of information in the human, further studies should be performed to fully evaluate the antitussive potential of tachykinin receptor antagonists.

Sigma receptor agonists have been associated with cough suppression. The most prominent of these is the cough-suppressant drug dextromethorphan. Given that dextromethorphan binds to *N*-methyl-D-aspartate and sigma-1 receptors, the specificity of its action to suppress cough has not been clear until recently. Brown et al. (2004) recently showed that this drug acts at sigma-1 receptors to inhibit cough in the guinea pig. Other sigma-1 receptor agonists can inhibit cough as well (Chau et al. 1983; Brown et al. 2004). Carbetapentane, a sigma-1 receptor agonist that is approved for use in humans as an antitussive, inhibits cough in the cat (Talbot et al. 1975) and guinea pig (Brown et al. 2004). The role of sigma-2 receptors in the suppression of cough is less clear.

Various other receptors have been associated with cough suppression in animal models, such as non-opioid receptors (NOP-1) (Bolser et al. 2001; McLeod et al. 2001, 2004; Lee et al. 2006), cannabinoid receptors (Gordon et al. 1976; Jia et al. 2002; Morita and Kamei 2003; Patel et al. 2003), and dopaminergic receptors (Kamei et al. 1987a; Li et al. 2002). In humans, dopaminergic receptors appear to have no effect on irritant-induced cough in normal subjects (O'Connell 2002).

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Fig. 1.

Brainstem regions that have been specifically associated with the actions of antitussive drugs. *Outlined areas* on the left indicate regions of the brainstem in which cough-related neurones have been recorded. *Shaded areas* represent regions in which application of cough-suppressant drugs has inhibited cough. The model on the right shows second-order interneurons in pulmonary vagal afferent pathways. Pump cells mediate sensory information from pulmonary slowly adapting receptors and neurones designated “relay” mediate information from cough receptors and rapidly adapting receptors. The notation shows that although a number of different drugs may act on this population of neurones, receptor specificity only for tachykinin neurokinin-1 receptors has been demonstrated (Chen et al. 2003; Sekizawa et al. 2003). NTS nucleus of the tractus solitarius, rVRC rostral ventral respiratory column, cVRC caudal ventral respiratory column