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Parasympathetic Control of Airway Submucosal Glands: Central Reflexes and the Airway Intrinsic Nervous System

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Abstract

Airway submucosal glands produce the mucus that lines the upper airways to protect them against insults. This review summarizes evidence for two forms of gland secretion, and hypothesizes that each is mediated by different but partially overlapping neural pathways. *Airway innate defense* comprises low level gland secretion, mucociliary clearance and surveillance by airway-resident phagocytes to keep the airways sterile in spite of nearly continuous inhalation of low levels of pathogens. Gland secretion serving innate defense is hypothesized to be under the control of intrinsic (peripheral) airway neurons and local reflexes, and these may depend disproportionately on non-cholinergic mechanisms, with most secretion being produced by VIP and tachykinins. In the genetic disease cystic fibrosis, airway glands no longer secrete in response to VIP alone and fail to show the synergy between VIP, tachykinins and ACh that is observed in normal glands. The consequent crippling of the submucosal gland contribution to innate defense may be one reason that cystic fibrosis airways are infected by mucus-resident bacteria and fungi that are routinely cleared from normal airways. By contrast, the *acute (emergency) airway defense reflex* is centrally mediated by vagal pathways, is primarily cholinergic, and stimulates copious volumes of gland mucus in response to acute, intense challenges to the airways, such as those produced by very vigorous exercise or aspiration of foreign material. In cystic fibrosis, the acute airway defense reflex can still stimulate the glands to secrete large amounts of mucus, although its properties are altered. Importantly, treatments that recruit components of the acute reflex, such as inhalation of hypertonic saline, are beneficial in treating cystic fibrosis airway disease. The situation for recipients of lung transplants is the reverse; transplanted airways retain the airway intrinsic nervous system but lose centrally mediated reflexes. The consequences of this for gland secretion and airway defense are poorly understood, but it is possible that interventions to modify submucosal gland secretion in transplanted lungs might have therapeutic consequences.

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8. Conclusion: Implications for cystic fibrosis and lung transplantation.

Introduction & Overview

The purpose of this review is to summarize evidence for and against a new view of the neural control of airway submucosal glands (Fig. 1) by the autonomic nervous system (Fig. 2). I hypothesize that airway glands participate in two distinctly different functions, each mediated by different levels of autonomic control and each depending to different extents on a separate set of neurotransmitters and ion channels.

Function one is Innate Mucosal Defense

Each day we inhale ~8,000 liters of air, which contains a variety of potentially harmful substances. Most of the time, airway glands participate in *innate mucosal defense*, the housekeeping function that keeps the airways clean and uninfected. In this process, the glands release small amounts of antimicrobial-rich mucus onto the airway surface that abets the continuous mucociliary clearance by the surface epithelium (Knowles and Boucher 2002) and the constant surveillance by lung phagocytes. The extent to which they do this continuously (spontaneous or basal secretion), and in response to neural signals stimulated by low levels of exogenous substances in the airways, is presently unknown. In either case it is important to identify the sources and nature of the routine, low level excitatory drive that operates during innate defense. The hypothesis to be considered is that *airway intrinsic neurons* and their associated neural networks in the airway walls play a large role in the housekeeping functions of airway innate defense, and that in this mode the glands are somewhat independent from central control. (Airway intrinsic neurons, with cell bodies and axons, are distinguished from *pulmonary neuroendocrine cells*. These latter cells are more extensive in human airways than previously appreciated (Weichselbaum, Sparrow et al. 2005), but their relation to the systems discussed here is unknown, and they will not be discussed further.).

Function two is the Emergency Airway Defense Reflex

During emergencies such as inhalation of water, irritating aerosols or large foreign bodies, the *emergency airway defense response* is triggered. This reflex consists of glottal closure, airway constriction, pulmonary vessel dilation, cough and copious gland secretion. The emergency airway defense response is centrally mediated and depends upon intact vagal connections to the lung. It is also strongly signaled to conscious awareness, and volitional control can to some extent supervene in the course of the response, as when individuals choose to prolong or suppress coughing in different circumstances (Hutchings, Morris et al. 1993). The glandular component of the emergency reflex is mediated primarily by cholinergic input to the glands, and involves high levels of fluid and mucin secretion accompanied by myoepithelial cell contractions. Importantly, the emergency response continues to operate in cystic fibrosis, although secretion rates to maximal levels of pharmacological stimulation appear to be reduced. It is entirely understandable that this robust response is what most gland physiologists have studied, but I will try to make the case that its relevance to cystic fibrosis may be the opposite of what has been claimed, and that the carefully controlled evocation of the emergency airway defense reflex may be therapeutic for people with cystic fibrosis.

In sum, it is apparent that glands participate in two kinds of function: the housekeeping function needed for routine protection of the airways from near-constant inhalation of low levels of pathogens, pollen, spores etc, and emergency responses to acute and drastic insults to the airways. The question to be addressed in this review is whether these functions are best thought of as a continuum, or as different functions with distinctive forms of neural control.

2. Airway Mucus & Submucosal Glands

Mucus

Starting within the nasal cavity and extending through all airways down to diameters of 1-2 mm, submucosal glands are present in humans and most large mammals (Fig. 1). In humans their density is roughly one gland per mm² of airway. In healthy airways the mass of the glands is at least 20-fold greater than the mass of surface goblet cells (Lamb and Reid 1972).

Furthermore, when mucus was collected from airways before and after removal of the surface epithelium, there was little difference in the amount collected (Trout, Corboz et al. 2001); these results suggest that glands supply ~95% of the mucus that keeps the airways sterile. The importance of airway glands to innate defense has been shown directly by experiments in which ferret tracheal xenografts with glands were much more resistant to infections than those that lacked glands (Dajani, Zhang et al. 2005).

Mucus is a sophisticated mixture of huge gel-forming mucins, diverse antimicrobials, anti-inflammatory molecules and immune cells, all in a salt solution of controversial composition. In addition to giving mucus its characteristic viscoelasticity, mucins contain a vast number of highly variable carbohydrate side chains that provide a “combinatorial library” of binding sites for pathogens (Knowles and Boucher 2002). These properties make it extremely difficult for any bacterium to penetrate healthy mucus. Indeed, in poorly controlled cystic fibrosis lung disease, where the lungs carry a heavy burden of bacteria, the bacteria are found in the mucus of the airway lumen and not bound to airway surface cells (Worlitzsch, Tarran et al. 2002).

The antimicrobials in mucus are also highly sophisticated, and they inactivate or kill bacteria by such a diverse array of mechanisms that it appears to be next to impossible for bacteria to evolve resistance to natural airway defenses. Based on bands observed in protein gels and on mass spectrometry of proteins from pure gland mucus, it is estimated that the gland serous cells produce >100 different compounds, most believed to be antimicrobials and anti-inflammatory substances (Joo, Lee et al. 2004). The most abundant of these is *lysozyme* (10-20 mg secreted per day), which breaks down the cell walls of bacteria by catalyzing the hydrolysis of β -1,4-glycosidic bonds between *N*-acetylmuramic acid and *N*-acetyl-D-glucosamine (Duszyk 2001). Glands also secrete the iron-binding protein *lactoferrin*, as well as *siderocalin*, (Joo, Lee et al. 2004), which binds bacterial catecholate-type ferric siderophores with subnanomolar affinity (Goetz, Holmes et al. 2002). The binding of siderophores will complement iron sequestering by lactoferrin to starve bacteria of iron and is potentially much more efficient because it only targets iron that has already been bound for bacterial use. Mucus also contains pore-forming defensins, and lactoperoxidase (Gerson, Sabater et al. 2000). Finally, sputum contains natural killer cells, cytotoxic T cells, macrophages and neutrophils, whose importance in airway innate defense has been repeatedly reviewed (Cole and Waring 2002; Ganz 2004; Beisswenger and Bals 2005).

Airway submucosal glands

The best structural information on human airway submucosal glands is based upon serial sectioning and electron microscopy. (Meyrick, Sturgess et al. 1969; Meyrick and Reid 1970). The original abstract can't be beat for conciseness: “A graphic reconstruction has been made of a submucosal gland from a normal human main bronchus, revealing a collecting duct not previously described. Ciliated respiratory epithelium dips into the gland opening to line the first part of the duct, the *ciliated duct*, and then gives way, in the *collecting duct*, to an epithelium composed of tall, columnar, eosinophilic cells containing numerous large mitochondria. This cell structure suggests that the collecting duct controls ionic and water concentration. From the collecting duct arise secretory tubules lined by mucous cells—*mucous tubules*. Tubules lined by serous cells—*serous tubules*—arise from mucous tubules either terminally or

laterally.” (Meyrick, Sturgess et al. 1969), (*italics added*). The existence of four distinct epithelial compartments within a single gland means that the mucus emanating from the duct orifice is a complex product of multiple cell types; the spatial separation of the cell types is interesting and not yet explained. In addition to the epithelial cells described by Meyrick and Reid, the mucous and serous tubules are covered with myoepithelial cells, which, by analogy with other glands (Emmelin, Garrett et al. 1977), provide when contracted a supporting structure against the hydrostatic pressure developed by epithelial fluid secretion.

The distribution of airway glands within airways and across species has been well studied by Widdicombe and his colleagues (Choi, Finkbeiner et al. 2000; Widdicombe, Chen et al. 2001; Widdicombe and Pecson 2002). A key point to emerge from these studies is that the density of glands *per unit area of airway surface* is highest in large airways and then declines linearly with airway diameter, essentially disappearing at airway diameters of ≤ 2 mm. Large airways have turbulent airflow that greatly increases the impaction rate on the airway walls, and Widdicombe suggests that this accounts for the relation. It can also account in part for the paucity of glands in small species like the mouse, but another key difference that explains species variation in gland density is the extent to which animals are obligate nose-breathers. Rabbits, for example, lack glands in their tracheas, but have a high density of glands in their extensively developed nasal cavities.

Gland secretion in response to pharmacological mediators has been extensively studied in the tracheas and isolated airways of pigs (Inglis, Corboz et al. 1997; Inglis, Corboz et al. 1997; Inglis, Corboz et al. 1998; Trout, Gatzky et al. 1998; Trout, King et al. 1998; Ballard, Trout et al. 1999; Crews, Taylor et al. 2001; Trout, Corboz et al. 2001; Phillips, Hey et al. 2002; Phillips, Hey et al. 2002; Phillips, Hey et al. 2003; Trout, Townsley et al. 2003; Ballard and Inglis 2004; Ballard, Trout et al. 2006). Control experiments establish that secretions studied in this way are virtually all from the submucosal glands. These experiments have established that the submucosal glands respond to acetylcholine and to forskolin, with the response to forskolin being less than half that to carbachol. Secretion is mediated by both chloride and HCO_3^- secretion, and involves CFTR, the anion channel that is defective in cystic fibrosis. When anion transport was inhibited, glands continued to secrete macromolecules which collected in the ducts and on the airway surface as a viscous material. This situation has some similarity to cystic fibrosis, although we will show that gland secretion to cholinergic mediators is partly spared in cystic fibrosis.

Secretion from individual glands was first studied by Quinton, who coated the surface of cat tracheas with oil and then quantified the mucus bubbles that form under the oil by collecting them with constant bore microcapillaries, allowing the first accurate measures of single gland airway gland secretion rates and the ion content of pure gland mucus (Quinton 1979). This method doesn't work well with sheep, pigs or humans, which have mucus that is much thicker than that of the cat (unpublished observations). However, the bubbles of mucus that form under oil in these species can be quantified optically, and single gland secretion rates for these species have been determined for a variety of mediators and conditions, and their pharmacology further studied (Jayaraman, Joo et al. 2001; Joo, Krouse et al. 2001; Joo, Wu et al. 2001; Joo, Irokawa et al. 2002; Joo, Saenz et al. 2002; Verkman, Song et al. 2003; Thiagarajah, Song et al. 2004; Salinas, Haggie et al. 2005; Joo, Irokawa et al. 2006; Song, Salinas et al. 2006). Among the findings of such studies are that vasoactive intestinal peptide (VIP), usually considered to be a muscle relaxant, is a good secretagogue for fluid secretion from pig and human airway glands, as was first suggested based on measures of lactoferrin release (Baraniuk, Lundgren et al. 1990). It has also been discovered that low (nM) levels of VIP and carbachol act synergistically to stimulate fluid (whole mucus) secretion (Choi, Ianowski et al. 2006; Choi, Joo et al. 2006; Choi, Joo et al. 2006), as was shown long ago by Shimura for glycoconjugate secretion from isolated cat glands (Shimura, Sasaki et al. 1988). Based on all of the studies to

date, as well as studies of the Calu-3 cell line (Finkbeiner, Carrier et al. 1993) that is used as a model of serous cells e.g. (Haws, Finkbeiner et al. 1994; Shen, Finkbeiner et al. 1994; Lee, Penland et al. 1998; Devor, Singh et al. 1999; Irokawa, Krouse et al. 2004), a model of gland secretion has been proposed in which VIP stimulates primarily HCO_3^- -mediated secretion that is dependent upon CFTR, while ACh stimulates primarily Cl^- -mediated secretion that does not require CFTR. Thus, secretion by glands to saturating levels of VIP or forskolin (Joo, Irokawa et al. 2002), as well as the synergistic increase of gland secretion to combinations of VIP + carbachol, are lost in cystic fibrosis (Choi, Joo et al. 2006; Janowski, Choi et al. 2006). In contrast, secretion to cholinergic agonists remains robust, although the level of secretion is reduced and the composition is altered (Jayaraman, Joo et al. 2001; Thiagarajah, Song et al. 2004; Salinas, Haggie et al. 2005; Song, Salinas et al. 2006).

What is the physiological significance of these observations for cystic fibrosis airway disease? The answer to that question requires us to understand how gland secretion is normally controlled by the autonomic nervous system.

3. The Airway Intrinsic Nervous System

It was long thought that “The gut is the only organ that is capable of manifesting reflex activity in the absence of input from the brain or spinal cord” (Kirchgeßner, Liu et al. 1996), citing earlier references. However, peripheral ganglia and nerves in the trachea were described as early as 1885 (Landois 1885) cited by (Fisher 1964), and the evidence to be summarized below, gathered by many investigators over at least the last two decades, indicate that the tracheal neurons form an *airway intrinsic nervous system* that helps control airway muscle tone, gland secretion and blood flow. Although not nearly as extensive as the intestinal enteric nervous system and not nearly as well studied, the importance of this network (and its limitations) is evident in people who have lung transplants. Transplanted lungs have lost their connections with the autonomic nervous system, and yet retain the ability to maintain the patency of the airways for many years in the best cases. It will be apparent as we proceed that while the classically described cholinergic, vagal postganglionic neurons are an important component of the airway intrinsic nervous system, their ‘relay’ role is abetted by excitatory interconnections among themselves, as well as with other, noncholinergic neurons. Importantly, the noncholinergic neurons mainly express VIP, and while the role of VIP in muscle relaxation is usually emphasized, our interest is in the function of VIP as an airway gland secretagogue.

Anatomy: Overview of airway intrinsic neural networks

When tracheas and bronchi are studied with methods designed to reveal neurons and neural processes, complex neural networks (nerve plexuses) with anatomically distinct regions are revealed (Fisher 1964). The first study in which a comprehensive view of the entire tracheal innervation was attempted was in ferrets (Coburn 1984; Baker, McDonald et al. 1986), and similar methods have been applied to mice (Chiang and Gabella 1986), dogs (Yamamoto, Ootsuka et al. 1998), guinea pigs (Baluk and Gabella 1989), cats (Kuder, Szczurkowski et al. 2003) and rats (Kusindarta, Atoji et al. 2004). An overview of the nerve networks in the ferret trachea is shown in Fig. 3. This was based on staining for acetylcholinesterase activity, which is also found in some non-cholinergic neurons (and in non-neural cells). The fibers stained include both those from the intrinsic neurons and the sensory and motor axons of the vagus, and probably included at least some non-cholinergic neurons.

The nomenclature is confusing, and the following description is a composite based on all of the studies, with a special debt to Yamamoto *et al.*, whose work on the dog trachea used the neuronal marker PGP 9.5, and identified five different neural plexuses starting with the

adventitia and progressing toward the mucosa (Fig. 4). The key point is that all of the cell bodies stained in the ferret and most of the neuronal cell bodies seen in the dog are found in the adventitia surrounding the trachea, especially over the dorsal region, which lacks cartilage and contains the *trachealis* muscle. *In situ*, this region would face the ventral esophagus; it receives vagal input from the recurrent laryngeal nerve, and in the ferret it contains distinctive, longitudinally oriented nerve tracts along which the largest ganglia with the largest neurons are found. These have been called **ganglia of the longitudinal nerve trunks** (Dey, Mayer et al. 1993).

Baker *et al.* distinguish two additional components of the peritracheal plexus: a superficial muscle plexus and superficial gland plexus. These two designations indicate the location of ganglia around the circumference of the trachea: that is, whether they were found over the trachealis muscle or were more lateral and ventral (Fig. 3, 5). The two sets of ganglia do not differ from one another either in the mean number of neurons they contain (1-4) or the size of their somata (24 μm in ferret), but they do differ markedly in both of these respects from the larger and more numerous trachealis motor neurons. They also differ with respect to the most obvious terminations of their axons: those in the superficial muscle plexus contributing to the innervation of the trachealis muscle (these terminals are called the deep muscle plexus) or the glands (these terminations are called the deep gland plexus).

To summarize results for the ferret, the acetylcholinesterase (AChE)-positive peritracheal nerve plexus located in the adventitia surrounding the trachea was found to contain ~4,000 neurons, ~28% of which are large cells within the ganglia of the longitudinal nerve trunks, while the remaining 72% are smaller neurons in smaller ganglia (including 18% that are found as single neurons). In terms of surface area, Baker *et al.* counted 404 neurons/cm² of tracheal adventitial surface. They estimated 845 neurons/cm² over the trachealis muscle (dorsal surface) and 202 neurons/cm² over the rest of the tracheal surface (Baker, McDonald et al. 1986). Airway submucosal gland densities in larger species are about 100/cm² of mucosal surface. Thus, ignoring the discrepancy between adventitial and mucosal surface area, ferrets could have as many as 6 neurons per gland if all non-longitudinal tract neurons innervated glands, and as few as 2 per gland if only the cells in the superficial gland plexus are secretory-motor cells.

Turning to the dog, several differences emerge, and it is impossible at present to determine which of these arise from species or methodological differences. To start, the intrinsic nervous system serving the dog trachea contained on average 18,461 neurons (range 13,902–24,232 in five dogs), or ~4-fold more than the ferret. Second, the intrinsic nervous system of the dog included neurons deep within the tracheal wall in layers termed the outer submucosal, inner submucosal and mucosal plexuses (Fig. 3). Neurons in these deeper regions were observed innervating glands (Fig. 3). Unfortunately, the number and sizes of neurons in these deeper plexuses was not given, but they occur 'often' in ganglia of 1-3 neurons in the submucosal layers but 'rarely' in the mucosal plexus.

Intrinsic neuron cell counts in the other species were 750 (2/mm²) in the rat (Kusindarta, Atoji et al. 2004), 235 in mouse (Chiang and Gabella 1986), and 222 in the guinea pig (Baluk and Gabella 1989). For cats no neural count was given, but there were 95-210 ganglia each with from 2-25 neurons (Kuder, Szczurkowski et al. 2003). In the guinea pig trachea, a somewhat simpler arrangement of ganglia and associated neural plexuses has been described based on staining for AChE activity, it contained only 222 neurons on average (Baluk and Gabella 1989). This unexpectedly small number may arise because of an important species difference: VIP and nitric oxide synthetase (NOS) neurons innervating the trachealis muscle of the guinea pig are not in the trachea, but instead originate in the esophagus (Fischer, Canning et al. 1998). Two conclusions seem justified: larger species tend to have more neurons in their airway

intrinsic neural networks, and great variability occurs among individual members of a species. The variability in neuron numbers, the small number of controlling vagal preganglionic neurons, and evidence that postganglionic neurons innervate their end organs with varicosities that are 20 nm to 2 μ m distant and do not form clear synapses (Burnstock 1988) suggest a somewhat diffuse level of control in this system.

Physiology: Properties of intrinsic airway neurons and evidence for interconnections among them

Intracellular recording methodology selects for larger neurons. Hence, the most thoroughly studied airway neurons are the large neurons closely associated with the longitudinal nerve trunks in ferrets (Coburn and Kalia 1986), and the presumably corresponding neurons in rat pups (Allen and Burnstock 1990), the peribronchial nerve ganglia in guinea pigs (Myers and Udem 1993; Myers and Udem 1995; Myers, Udem et al. 1996; Myers and Udem 1996; Myers 2000; Kajekar, Rohde et al. 2001; Myers 2001; Canning, Reynolds et al. 2002; Myers, Goldie et al. 2005), and corresponding neurons in cats (Mitchell, Herbert et al. 1987), and humans (Kajekar, Rohde et al. 2001). LGT neurons are multipolar, and their processes (sometimes two or three axons) run in the longitudinal nerve trunks (Fig. 5A.) where they appear to synapse with one another as well as innervating the trachealis muscle (Coburn and Kalia 1986).

In a unique and especially informative study, the presumed counterparts of these neurons in cats (mean soma diameter of 63 μ m and projections to the tracheal smooth muscle) were studied *in vivo* with intracellular microelectrodes, and were shown to fire during the inspiratory phase of breathing as a result of summated synaptic input; the trachealis muscle also contracted during inspiration (Fig. 6.) The trachealis motor neurons were silent during the expiratory phase, as a result of diminished excitatory input rather than inhibition. The primary source of the excitation was judged to be descending input from vagal preganglionic neurons, which excite the cells via nicotinic cholinergic synapses (Mitchell, Herbert et al. 1987). This is a remarkable finding with numerous implications for understanding the routine operation of airways physiology, but to my knowledge it has never been pursued. As is also shown in Fig. 6, a different set of intrinsic neurons was excited during the expiratory phase. While not neurochemically identified in that study, these neurons innervate the glands, and their anatomical properties suggest that they may be the counterpart of the VIP/Substance P/NOS neurons of the ferret (Dey, Altemus et al. 1996; Zhu and Dey 2001).

Although the rhythmicity observed in the cat was correlated with respiratory movements and hence attributed to descending excitatory drive, complex (bursting) synaptic activity has also been observed in airway ganglion cells recorded in *isolated* tracheas of the rat *in vitro* (Fig. 6) (Allen and Burnstock 1990). The ganglion cell EPSPs were eliminated by hexamethonium, and reduced but not eliminated by TTX. While some of the EPSPs probably come from spontaneous release from the cut vagal efferents, others almost certainly arise from ACh being released by the terminals of airway intrinsic neurons. Thus airway intrinsic neurons, in addition to stimulating muscles and glands directly via muscarinic (M3) metabotropic synapses, also can stimulate them indirectly via axon collaterals to other ganglionic cells: these operating by nicotinic, ionotropic receptors (Fig. 6C, D). [The larger neurons (LGT neurons in ferrets) rarely express either VIP or NOS (Dey, Mayer et al. 1993)].

In the only electrophysiological study done of *human* airway intrinsic neurons (Fig. 6E, F), phasic and tonic neurons were distinguished. Phasic cells had shorter time constants, and produced a single impulse to sustained depolarizing current. In contrast, tonic neurons, fired repetitively with increased frequencies as the depolarization was increased (Kajekar, Rohde et al. 2001). Synaptic input from stimulation of the supplying bronchial nerve produced fast

EPSPs mediated by nicotinic ACh receptors. These did not reach threshold in half the neurons studied in spite of attempts to stimulate all inputs maximally.

Airway intrinsic neurons expressing VIP, NOS and SP

Nerve fibers containing VIP innervate airway smooth muscles, glands and blood vessels (Uddman, Alumets et al. 1978; Dey, Shannon et al. 1981), and VIP is found in airway intrinsic cell bodies (Uddman, Alumets et al. 1978; Dey, Hoffpauir et al. 1988). Substance P (SP) was first localized in airway sensory nerves (Lundberg, Hokfelt et al. 1984) and was later colocalized with VIP in fibers innervating glands and smooth muscle and was shown to be expressed by airway intrinsic neurons (Dey, Hoffpauir et al. 1988). After nitric oxide synthase (NOS) was localized to neurons and the concept of nitric oxide (NO) as a neural transmitter advanced (Bredt, Hwang et al. 1990), NOS was also localized to airway nerves and airway intrinsic nerve cell bodies (Dey, Mayer et al. 1993). Thus, by 1993, intrinsic neurons containing VIP, SP and NOS were known to be present in the airways. Intrinsic airway neurons expressing VIP, SP or NOS have been studied in rats (Fontan, Cortright et al. 2000; Van Genechten, Brouns et al. 2003), cats (Dey and Zhu 1993), guinea pigs (Fischer, Canning et al. 1996), ferrets (Dey, Mayer et al. 1993; Dey, Altemus et al. 1996; Wu, Maize et al. 2001; Wu and Dey 2006), and humans (Fischer, Canning et al. 1996). No studies of VIP-expressing intrinsic airway neurons have been carried out in pigs, in spite of the extensive use of pigs as models of human airway function.

It is not presently possible to trace immunohistochemically labeled axons and terminals to their cell bodies of origin. Thus, to establish the proportion of VIP and SP axons originating from airway intrinsic neurons, pieces of cat airway were maintained in organ culture for 7 days, so that only processes from neurons with cell bodies within the isolated piece of tissue remained (Dey, Altemus et al. 1991). Three important findings emerged from this study. First, SP, previously thought to originate exclusively in sensory cells with cell bodies in the nodose ganglion, was also found to be expressed by airway intrinsic neurons; second, many intrinsic neuron cell bodies express VIP, and VIP terminals were observed around neurons, glands and blood vessels, and third, SP terminals, which are prevalent in the epithelium of freshly isolated airways, disappear in the cultured airways, suggesting that the mucosal SP terminals are all derived from distant sensory cell bodies. The presence of intrinsic nerve terminals around intrinsic nerve cell bodies is especially important because it provides further evidence that functional neural networks are intrinsic to the airways.

The neurochemical profiles of the intrinsic neural network in the ferret trachea were revealed by multiple-labeling of intrinsic neurons for choline acetyltransferase (ChAT), NOS, VIP, and SP (Dey, Altemus et al. 1996). About 85% of the ChAT-positive neurons were located in longitudinal trunk ganglia or the closely associated bridge ganglia, while 15% were in ganglia of the superficial muscular plexus. More than 75% of VIP-immunoreactive neurons were in the superficial muscular plexus with <10% in the longitudinal trunks or bridge neurons; most NOS and SP neurons were also located in the superficial muscular plexus. The transmitter profile for neurons of the superficial muscular plexus was: 11% only NOS, 20% only VIP, 5% only SP, 67% NOS and VIP, and 40% VIP and SP. NOS, VIP, and SP were frequently localized in the same nerve cell body. The occurrence of nerve terminals containing only SP located around the borders of individual NOS/VIP/SP-containing neurons suggests possible sensory innervation to the airway neurons. The results demonstrate that: (1) most cholinergic nerves do not contain VIP, NOS, or SP; (2) cholinergic neurons are predominantly located in the longitudinal trunk ganglia; (3) VIP, NOS, and SP are predominantly located in the superficial muscular plexus ganglia; and (4) nerve terminals containing exclusively SP, suggesting a possible sensory function, are closely associated with some neurons in the plexus. It is well to keep in mind that the terms used to identify the location of cell bodies are anatomical and not

functional terms. Thus, some VIP-containing neurons in the superficial muscular plexus send projections to the epithelium, where they run mainly parallel to the basement membrane at the base of the epithelial cells (Dey, Satterfield et al. 1999). These results from ferrets have their counterparts in humans, where the same three neurotransmitters are also found within intrinsic airway neurons (Fischer, Canning et al. 1996; Fischer and Hoffmann 1996).

Relations among cholinergic and VIP/NOS neurons

In a beautiful study combining neural tracing and neurotransmitter identification, longitudinal tract (LT) ganglia within the airway intrinsic nervous system of the ferret trachea were injected with the neural tracers fluororuby (FR) or biotinylated dextran-amine (BDA), which were taken up and transported in both anterograde and orthograde directions—tracheas were cultured for up to 2 days to allow for transport (Zhu and Dey 2001). In addition to confirming the interactions among neurons in the longitudinal tracts seen in earlier studies, this work revealed that the cholinergic LT neurons are presynaptic to VIP- and nNOS-containing neurons in the superficial muscular plexus (Fig. 7), and conversely that the SMP neurons send axons back to the LT ganglia. Some of the labeled neurons sent terminals to the trachealis muscle and others to other neurons, suggesting the possibility of true interneurons in this system in addition to efferents. What isn't clear from this study (or any study that I'm aware of) is whether vagal brainstem preganglionic neurons synapse directly on the VIP- and NOS-containing neurons. This work provides some of the most convincing (and esthetic) evidence that the neurons in the tracheal plexus form a functional network.

As with virtually every other study of VIP- and nNOS-containing airway neurons, the focus here was on the role of these mediators in producing muscle relaxation, but to repeat: VIP stimulates gland secretion. Nitric oxide would be expected to enhance gland secretion *in vivo* by increasing blood flow to the glands.

4. Gland Participation in Innate Defense

Innate defenses are ever-vigilant to low levels of inhaled pathogens. Here we address three questions: do airway glands participate in this “24-7” mode of airway defense? If so, do they do so in a regulated way or do they simply secrete a basal level of mucus at all times? Finally, if they are regulated, what are the relative roles of central and intrinsic neural control?

The possibility that airway innate defense does not rely on glands, or does so to a trivial degree, was tested in a powerful xenograft experiment by Engelhardt and his colleagues (Dajani, Zhang et al. 2005), in which ferret tracheas were excised and implanted into nude mice in such a way that the lumen was accessible for studies of bacterial killing. These were compared with denuded rat tracheas onto which a ferret epithelium was reconstituted. The reconstituted epithelium is essentially indistinguishable from that in the trachea having intact glands, allowing a direct assessment of the glandular contribution to airway defense. The findings were clear: 1) secretions harvested from the tracheas with glands killed bacteria much better than those with only the surface epithelium; 2) instillation of bacteria into the tracheas *in vivo* again showed a marked advantage for the trachea with glands in terms of bacterial killing, and 3) substantial amounts of lysozyme were found in the secretions of the tracheas with glands, but not in those having only epithelia. These studies provide direct evidence for the long-held assumption that airway glands are a critical component of the innate defenses of airways.

Equally important in terms of the questions posed here is that *the functional glands in the tracheal xenograft received no vagal innervation*. No assessment was made of intrinsic neurons in these experiments, and so one can't decide between the possibilities that glands have an intrinsic level of basal secretion or that gland secretion is stimulated by intrinsic neural circuitry.

As we saw in the previous section, intrinsic airway neurons, at least in the ferret, comprise two neurochemical populations. The large cell longitudinal tract neurons are primarily cholinergic, while the smaller neurons in the superficial plexuses primarily contain VIP, often co-localized with SP and NOS. These findings have been perplexing for students of smooth muscle physiology, but they make good functional sense in terms of recent experiments with airway glands. Glands are heavily innervated by VIP, SP and NOS-containing nerve terminals. Glands secrete well in response to VIP, not only glycoconjugates as once thought (Peatfield, Barnes et al. 1983), but also fluid (Joo, Irokawa et al. 2002; Joo, Saenz et al. 2002). They also respond to SP (Coles, Neill et al. 1984; Gashi, Borson et al. 1986; Shimura, Sasaki et al. 1987; Haxhiu, Haxhiu-Poskurica et al. 1990; Davis and Tseng 1991; Wagner, Fehmann et al. 1995; Trout, Corboz et al. 2001), and at low concentrations SP greatly augments the secretory rate produced by VIP (J. Y. Choi, J. P. Ianowski, J. W. Hanrahan, J. J. Wine, *unpublished observations*). The effect of NO has not yet been tested on single gland secretion, but it does not release glycoconjugates from airway explants (Kim, Okamoto et al. 2006), suggesting that its role in gland secretion might be to increase blood flow around the glands in support of fluid transport.

Clearly, the glands have substantial innervation from VIP and SP-containing neurons in the airway intrinsic nervous system. What drives these neurons? The study by Mitchell *et al.* was cited earlier as showing rhythmic vagal input to LGT neurons. As part of that study, the presumed counterparts of the VIP/NOS/SP neurons (cell bodies of 36 μm vs. 63 μm for the LGT cells) were also studied *in vivo*, and were shown to fire during the *expiratory* phase of breathing (Fig. 6B). Injection of the cells in the cat showed axons coursing toward the glands, and although they could not be traced to their terminations, it seems likely that these provide VIP and SP innervation to the glands (Coburn and Kalia 1986). These remarkable results suggest that airway glands, at least in the cat, receive rhythmic excitatory input during breathing. The input to the postganglionic neurons going to the trachealis muscle is almost certainly derived from airway vagal preganglionic neurons (AVPNs) that fire during the inspiratory phase of breathing (Widdicombe 1966). No such source of vagal excitation has been described for the expiratory phase of breathing, and it is possible that the source of at least some of this input arises is from the airway intrinsic neural circuits (Allen and Burnstock 1990). Nevertheless, these results suggest at least some level of tonic central drive to the airway glands, and this drive will increase in intensity during high rates of breathing (Coburn and Kalia 1986).

A final possibility is that, in addition to a low level of general, tonic secretion, airway glands may increase their secretion of mucus in local regions of the airway in response to signals from the mucosa caused by the deposition of pathogens or irritants. Presumably, local responses would be mediated by intrinsic circuitry of the kind we have been considering, and it now seems evident that abundant sensory input reaches these local circuits from C-fibers located in the epithelium. Recent evidence suggests that these local circuits can continue to stimulate gland secretion in isolated trachea and bronchi (Ianowski, Choi et al. 2007). Most afferents, and possibly all of them, have central cell bodies and so would cease to operate after denervation. However, the role of airway intrinsic neurons containing SP is unknown—these and possibly other intrinsic neurons could conceivably play a sensory role.

In summary, the airway innate defense system works continuously to prevent lung infections, and glands clearly participate in innate defense. However, unlike the emergency reflex system we will consider next, gland participation in innate defense has rarely been studied, and therefore much less is known about how glands function in this role. Key points to be determined are the extent to which gland participation in innate defense depends upon the intrinsic neural circuitry of the airways (critical for lung transplant patients) and on VIP innervation (critical for cystic fibrosis patients, whose glands no longer respond to VIP (Joo, Irokawa et al. 2002)). It will also be important to establish the extent to which gland secretion

of antimicrobials operates more-or-less continuously (a prophylactic function) and the extent to which gland secretion can increase following local deposition of pathogens or irritations. A role for the latter function is indicated by experiments in which gland secretion is triggered by very low levels of VIP and SP or ACh acting together (Choi, Ianowski et al. 2006; Choi, Joo et al. 2006; Choi, Joo et al. 2006), and by experiments in which the glands respond to local irritation of the mucosa in the absence of central neural control (Ianowski, Choi et al. 2007).

5. The Emergency Airway Defense Reflex

The *emergency airway defense reflex* is centrally mediated via a reflex pathway of vagal afferents and efferents and their coordinating brainstem nuclei (Fig. 2 and Fig. 8). It comprises, to various degrees and order: glottis closure, cough, airway constriction, apnea, rapid shallow breathing, vasodilation leading to increased airway vessel blood flow (especially to the mucosa) and submucosal gland secretion (Newhouse, Sanchis et al. 1976; Widdicombe 1977; Nadel, Davis et al. 1979; Schultz, Roberts et al. 1985; Pisarri, Jonzon et al. 1992; Pisarri, Coleridge et al. 1993; Coleridge and Coleridge 1994). The reflex protects the airways from acute inhalation of substances that are immediately injurious to the lungs. Apparent conflicts among reflex components arise because they are typically studied one at a time in anesthetized or decerebrate animals; conflicts disappear in the coordinated responses of active animals.

Anatomy

The reflex pathway is thought to have a minimum length of at least 4 neurons: 1) vagal afferents (~10% myelinated and 90% unmyelinated) arising from cells in the nodose/jugular ganglia (treated in more detail in the next section) synapse on 2) interneurons in the *nucleus of the solitary tract* (the brain stem's major ANS coordinating region), which in turn synapse (perhaps via polysynaptic pathways) onto 3) airway vagal preganglionic neurons (AVPNs) which are mainly in the *nucleus ambiguus* but also in the *dorsal motor nucleus of the vagus* and which send myelinated axons in the vagus nerve to synapse on 4) airway parasympathetic postganglionic neurons which in turn synapse on 5) muscles, glands and blood vessels.

The synaptic actions in the reflex pathway are as follows: 1) afferents release their respective neurotransmitters (this is glutamate when the reflex is triggered by lung deflation) (Haxhiu, Kc et al. 2005) 2) neurons of the *nucleus of the solitary tract* also release glutamate that acts on AMPA receptors expressed by the AVPNs; 3) the AVPNs release both VIP and ACh (Kc, Mayer et al. 2004). The target of the VIP is unidentified, but the ACh acts on nicotinic, ionotropic receptors on the airway postganglionic neurons, which in turn 4) release ACh that stimulates M3 muscarinic metabotropic receptors on muscles and glands. The AVPNs may be the bottleneck (point of maximum convergence) within the reflex pathway. The number of AVPNs innervating the airways in different species is not known with certitude, but it is a relatively small number. Human recurrent nerves to the trachea have ~8,000 axons per side, with ~2,800 of these being myelinated, and most of the myelinated axons are afferent, leaving a relatively small number of preganglionic efferents to control the trachea. The small number of AVPNs places constraints on degree of centrally-mediated independent action by different effectors (muscles, glands and blood vessels) and by different regions of the airways.

The simple serial pathway outlined above is the backbone of a more complex system that appears to involve both parallel feed-forward pathways and additional divergence, as follows: 1) sensory afferents can feed-forward to directly innervate airway intrinsic neurons and their effectors (axon reflexes). 2) the parasympathetic postganglionic neurons interconnect with one another so that input is conveyed over a larger spatial region, 3) postganglionic cholinergic neurons are in turn presynaptic to non-cholinergic airway intrinsic neurons (Zhu and Dey 2001), 4) there is circumstantial evidence, based on retrograde labeling of AVPNs with subunit B of cholera toxin (CTB) from sites in airway mucosa, that AVPNs might feed-forward to

effector targets within the airway wall, as is known to occur in other parasympathetically innervated targets (Perez Fontan and Velloff 2001;Kc, Mayer et al. 2004). However, see also (Atoji, Kusindarta et al. 2005).

Integrative physiology

Because the acute airway defense reflex is centrally mediated, its study requires the difficult and expensive methods of whole animal integrative physiology. The participation of the glands in the airway vagal reflex was studied early and extensively by Nadel and his colleagues, and the Coleridges and their colleagues using anaesthetized dogs and cats, and a method for measuring gland secretions by video imaging of 'hillocks' formed by mucus emerging from airway glands under a layer of tantalum powder. Nadel states the results succinctly: "The output of secretions from the airway submucosal glands is regulated by vagal efferent nerves. Stimulation of cough receptors increases mucus output reflexly via the vagus nerves" (Nadel, Davis et al. 1979). In a long series of studies, reflex gland secretion and other components of the defense response were shown to be stimulated by tactile stimulation of the larynx (German, Ueki et al. 1980), and by gastric irritation (German, Corrales et al. 1982), cigarette smoke (Schultz, Davis et al. 1991), and capsaicin and bradykinin injection into a bronchial artery (Davis, Roberts et al. 1982), all of which are known or thought to activate C fibers (Coleridge and Coleridge 1994). Gland secretion is also stimulated by decreases in lung compliance, which like tactile stimulation activates rapidly adapting receptors (RARs) but not C-fibers (Yu, Schultz et al. 1989), and by acute hypoxemia, detected by receptors in the carotid body (Davis, Chinn et al. 1982). These studies were followed up by Haxhiu and colleagues (Fig. 8) who also used the tantalum, hillock counting method to study reflexly stimulated airway gland secretion under a variety of conditions in anesthetized or decerebrate pigs (Haxhiu, Haxhiu-Poskurica et al. 1990) or dogs (Haxhiu, Cherniack et al. 1991;Haxhiu, van Lunteren et al. 1991;Haxhiu, Van Lunteren et al. 1991;Hejal, Strohl et al. 1993;Hejal, Strohl et al. 1995;Haxhiu, Chavez et al. 2000). To my knowledge, the research by Haxhiu and his colleagues [see (Haxhiu, Kc et al. 2005) for a review] is currently the *only* integrative experimentation being done anywhere on central control of airway gland secretion.

In the context of cystic fibrosis, it is of particular interest that the airway vagal defense reflex can be triggered by small amounts of either hypo- or hyperosmotic salt in the airway, with isotonic saline or glucose being ineffective. In experiments with anesthetized dogs, injections into a lobar bronchus of small amounts of water or hypertonic salt (4-8 %), but not isotonic salt or glucose, stimulated pulmonary and bronchial C-fibers and rapidly adapting receptors and caused bradycardia, arterial hypotension, apnea followed by rapid shallow breathing, and contraction of tracheal smooth muscle (Pisarri, Jonzon et al. 1992). Although gland secretions were not measured, they are known to accompany these kinds of responses.

This study was followed by one in which water was instilled in a bronchus while measuring blood flow to the airways (Pisarri, Coleridge et al. 1993). Increased blood flow is an excellent general surrogate for activation of the airway glands in the context of the defense response. Airway glands are densely vascularized, and increased blood flow to them helps support the secretory activity that is their contribution to airways defense. In this elegant and carefully described study, arterial blood flow was measured with an ultrasonic transit time probe, and colored microspheres were used to determine the regional distribution of blood flow and changes in flow in the tracheal wall. Water in a bronchus stimulated a 2-fold increase in blood flow bilaterally to the airways, without altering blood flow elsewhere; within the airway wall the largest blood flow was in the mucosa (where the glands are), rather than in the regions of muscles.

Among the strengths of this study is that individual differences among the dogs were reported, rather than just giving averages. It was noted that while increased blood flow was abolished

by vagal interruption in 13 of 16 dogs (81%), in 3 dogs (19%) a portion of the response persisted, measuring 17, 33 and 77% of the response with the vagus intact (Pisarrì, Coleridge et al. 1993). This evidence for locally mediated components of the response is important, but it would be easy to overlook in experiments that report only average responses. The inference that increased gland secretion was accompanying blood vessel dilation in these experiments is supported by a direct study of reflex activation of gland secretion that is notable for including three measures of the defense response: increase in airway muscle tension, increased blood flow, and gland secretion (Haxhiu, Chavez et al. 2000). All three responses were triggered in decerebrate dogs by lung deflation (Fig. 8).

6. Airway Receptors: Better Methods Reveal More Diversity

Airway receptors have traditionally been classified as Rapidly Adapting Receptors (RARs), Slowly Adapting Receptors (SARs) and C-fibers, and their properties have been extensively reviewed (Kubin, Alheid et al. 2006) and Table 1 in (Canning 2006). Many stimuli that elicit gland secretion are detected by the mucosal surface, and consistent with that, a dense plexus of primarily afferent nerves is present in the mucosa, perhaps 10 fibers per mm² in the human trachea (Laitinen 1985). If sufficiently stimulated, most classes of airway receptors can probably trigger emergency defense reflexes including gland secretion (Coleridge and Coleridge 1994). However, recent research is revealing more receptor specificity than the traditional tripartite classification, and this increased understanding of receptor specificity has considerable therapeutic potential.

For example, cough is readily triggered by even minor tactile stimulation of the carinal trachea, which activates myelinated, rapidly adapting receptors that are especially prevalent in that region and were called 'cough receptors' (Widdicombe 1977). The counterparts of these receptors have recently been identified in guinea pigs as a special class of myelinated receptor with unique properties, including sensitivity to punctate stimuli and to acid, insensitivity to capsaicin, and distinctive terminal arborizations within the mucosa. It is likely, although it was not reported, that these receptors also stimulate mucus secretion (Canning, Mazzone et al. 2004; Canning 2006; Canning, Farmer et al. 2006).

In guinea pigs the central vagal sensory afferents originate in two ganglia, the nodose and jugular, and in a series of elegant papers by Undem and his colleagues, significant progress has been made in sorting out vagal afferents by characterizing them according to their ganglion of origin. Virtually all of the tracheal epithelial terminals tested arose from *jugular C-fibers* that innervate the larynx, trachea and bronchus. About 60% of the jugular C-fibers with epithelial terminals expressed SP (Hunter and Undem 1999), and none of them expressed P2X receptors (Undem, Chuaychoo et al. 2004). The jugular afferents include C and A δ axons, and these respond differentially to capsaicin (mainly C fibers) and hypertonic saline (mainly A δ axons (Fig. 9). The A δ afferents that respond to hypertonic saline are of particular interest because inhalation of hypertonic saline is therapeutically beneficial for patients with cystic fibrosis (Robinson, Regnis et al. 1996; Elkins, Robinson et al. 2006), but the basis for this effect is unclear (Levin, Sullivan et al. 2006).

Nodose C-fibers primarily innervate structures within the lungs and are less likely to express neurokinins, but 100 % of them express P2X receptors in both their terminals and cell bodies as indicated by their responsiveness to α,β -methylene-ATP (Undem, Chuaychoo et al. 2004). Nodose sensory neuron terminals are not in the epithelium, but are labeled by dye in the submucosa (Hunter and Undem 1999). P2X receptors are ATP-sensitive cation channels with significant conductance for Ca²⁺ ions (Khakh and North 2006). Their role in signaling by airway sensory afferents is of great potential interest.

In summary, progress is being made in sorting out the properties of the parasympathetic sensory afferents that innervate the airways. These properties almost certainly have evolved to allow the afferents to carry out specific functions, yet most of the afferents can be stimulated by several stimuli, and their properties are quite plastic, changing quickly in response to inflammation, for example (see next section). During activation of the emergency airway defense reflex, it seems as if much of this specificity is over-ridden, because many different types of afferents are excited, and they in turn excite parasympathetic efferents which have diffuse effects. Because gland secretion to these reflex pathways is at least partially intact in people with cystic fibrosis, judicious activation of components of the reflex by carefully selected stimuli may be therapeutic.

7. Hijacking Emergency Defense for Innate Defense: Receptor Plasticity and Airways Sensitization

Almost everyone has suffered the aftermath of airway infections, when for days or weeks the airways produce copious mucus, and normally innocuous stimuli trigger coughing. The mechanisms underlying such sensitization are being intensely investigated because of their potential relevance to asthma and other forms of airway hyperreactivity. One level of sensitization may occur at the level of afferent interactions in the brain stem (Mazzone and Canning 2002), as well as peripherally—for a clear example see (Kajekar, Undem et al. 2003) and for a good overview see (Dey 2003).

This area won't be reviewed here, but it deserves its own section because of its relevance for the hypothesis being advanced. What is emerging from current studies is that agents that sensitize the airways can change the levels of neurotransmitters expressed by airway neurons (Dey 2003) and their electrophysiological properties e.g. (Kajekar, Undem et al. 2003) with the net effect of increasing synaptic transmission. These changes will produce lower reflex thresholds and stronger efferent activation of glands and muscles. Thus, when the airways are sensitized, stimuli that would normally be handled silently by the innate defense system may now trigger central defense reflexes, with all of their sequelae: cough, bronchoconstriction and increased gland secretion. While initially useful, the undue prolongation of this reflex state leads to morbidity; hence the considerable attention paid to it.

The main point in the context of this review is that the neural control of airway glands, which I have hypothesized to be under joint control of central reflex and peripheral control pathways, may well shift to more central control in sensitized airways.

8. Conclusions

Airways perform the mundane but essential function of conducting air to the alveoli where gas exchange occurs, and like other plumbing, they can be ignored unless they malfunction. The main theme that emerges from this review is that airway submucosal glands help protect the airways against both mundane, low level inhalation of pathogens (innate defense) and acute, potentially life-threatening aspiration of larger amounts of harmful substances (the emergency lung defense reflex).

The hypothesis that emerged during consideration of these dual roles proposes that there are dual levels of neural control serving each function (Fig. 10). Pathogens are usually dealt with by an unobtrusive innate defense system in which low level gland secretion, mediated in large part by the airway intrinsic nervous system, supplies small amounts of antimicrobial-rich mucus to the airway surface, where it is cleared by the mucociliary escalator. In contrast, the centrally-mediated vagal reflex primarily deals with inhalation of substances posing immediate

threats to the lungs. I have also hypothesized that these two different functions have evolved somewhat separate, although clearly overlapping systems of neural control.

Sustained Innate Defense Secretion

I hypothesize that the lower rates of gland secretion used for innate defense are primarily controlled driven by VIP-stimulated, cAMP-mediated and CFTR-dependent secretion; this form of secretion is hypothesized to rely in part on neural circuits intrinsic to the airways, and hence to persist in the absence of descending vagal excitation, although a tonic level of central excitation is present. To the extent that gland participation in this prophylactic and local-response innate defense system requires functional CFTR, it will be lost in cystic fibrosis, and this is hypothesized to be an important reason that mucus-resident bacterial infections are so readily acquired by cystic fibrosis airways. On the other hand, the partial independence of this pathway from central control means that it should continue to operate at some level in the denervated lungs of lung transplant patients.

Acute Emergency Defense Reflex Secretion

In contrast to the above system, I have summarized evidence that the high rates of gland secretion produced as part of the emergency defense reflex, which require intact vagal afferent and efferent reflex pathways through the brainstem, produce primarily an ACh-stimulated, $[Ca^{2+}]_i$ -mediated and CFTR-independent secretion. To the extent that this pathway is CFTR-independent, it will continue to function in CF airways, and there is clear evidence that it does so. Indeed, activation of CFTR-independent gland secretion by partial recruitment of the emergency defense pathways, as may happen, for example, with inhalation of hypertonic saline or strenuous exercise, appears to have beneficial consequences for CF airways. On the other hand, this type of gland secretion is lost in lung transplant patients, at least until reinnervation occurs. The consequences this may have for lung health deserve further study.

Why isn't the emergency system sufficient to prevent CF airway infections, and why don't infected and inflamed CF airways trigger this system vigorously to clear the airways? One possible answer is that the emergency system, while much more intact in CF than the essentially absent housekeeping system, is nevertheless partially compromised (Jayaraman, Joo et al. 2001; Thiagarajah, Song et al. 2004; Salinas, Haggie et al. 2005; Song, Salinas et al. 2006). Another possible answer is that while the emergency system may ameliorate CF symptoms, it is less effective because of the slow onset and chronic nature of CF infections, and because at least some aspects of it (e.g. cough) can be consciously overridden. For example, smokers learn to tolerate the noxious stimuli that initially trigger the defense reflex, and a similar process of adaptation may occur in CF. Coughing is unpleasant and sometimes socially embarrassing. There is anecdotal evidence that people with CF who are 'good coughers' do better, and productive coughing is a staple of good CF pulmonary hygiene. Issues like these, presently open only to speculation, could be transformed into testable hypotheses with the development of a true animal model of CF airways disease.

Reflexes are by definition graded in intensity, and the question arises whether the two types of gland control proposed here aren't simply the extremes of a continuum. Perhaps, but the many differences in mechanisms and control pathways outlined above suggest qualitative differences. If two systems do indeed exist, why have they not been differentiated before? One possibility is that larger responses are simply easier and more gratifying to study, and it is often assumed that maximal responses produced by pharmacological agents are scaled up versions of smaller ones. But many examples suggest otherwise. Among the more thoroughly documented accounts of different mechanisms being recruited by stimuli of increasing intensity is the story of calcium handling by pancreatic acinar cells, where stimulation of the kind often used in laboratory experiments leads to pancreatitis *in vivo* (Petersen and Sutton 2006).

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References

- Allen TG, Burnstock G. A voltage-clamp study of the electrophysiological characteristics of the intramural neurones of the rat trachea. *J Physiol* 1990;423:593–614. [PubMed: 2388159]
- Atoji Y, Kusindarta DL, Hamazaki N, Kaneko A. Innervation of the rat trachea by bilateral cholinergic projections from the nucleus ambiguus and direct motor fibers from the cervical spinal cord: a retrograde and anterograde tracer study. *Brain Res* 2005;1031(1):90–100. [PubMed: 15621016]
- Baker DG, McDonald DM, Basbaum CB, Mitchell RA. The architecture of nerves and ganglia of the ferret trachea as revealed by acetylcholinesterase histochemistry. *J Comp Neurol* 1986;246(4):513–526. [PubMed: 3700726]
- Ballard ST, Inglis SK. Liquid secretion properties of airway submucosal glands. *J Physiol* 2004;556(Pt 1):1–10. [PubMed: 14660706]
- Ballard ST, Trout L, Bebok Z, Sorscher EJ, Crews A. CFTR involvement in chloride, bicarbonate, and liquid secretion by airway submucosal glands. *Am J Physiol* 1999;277(4 Pt 1):L694–699. [PubMed: 10516209]
- Ballard ST, Trout L, Garrison J, Inglis SK. Ionic mechanism of forskolin-induced liquid secretion by porcine bronchi. *Am J Physiol Lung Cell Mol Physiol* 2006;290(1):L97–L104. [PubMed: 16183670]
- Baluk P, Gabella G. Innervation of the guinea pig trachea: a quantitative morphological study of intrinsic neurons and extrinsic nerves. *J Comp Neurol* 1989;285(1):117–132. [PubMed: 2474009]
- Baraniuk JN, Lundgren JD, Okayama M, Mullol J, Merida M, Shelhamer JH, Kaliner MA. Vasoactive intestinal peptide in human nasal mucosa. *J Clin Invest* 1990;86(3):825–831. [PubMed: 2203825]
- Beisswenger C, Bals R. Antimicrobial peptides in lung inflammation. *Chem Immunol Allergy* 2005;86:55–71. [PubMed: 15976488]
- Bredt DS, Hwang PM, Snyder SH. Localization of nitric oxide synthase indicating a neural role for nitric oxide. *Nature* 1990;347(6295):768–770. [PubMed: 1700301]
- Burnstock, G. Autonomic neural control mechanisms with special reference to the airways. *The Airways: Neural Control in Health and Disease*. In: Kaliner, MA.; Barnes, PJ., editors. *Lung Biology in Health and Disease*. 33. 1988. p. 1-22.
- Canning BJ. Anatomy and neurophysiology of the cough reflex: ACCP evidence-based clinical practice guidelines. *Chest* 2006;129(1 Suppl):33S–47S. [PubMed: 16428690]
- Canning BJ, Farmer DG, Mori N. Mechanistic studies of acid-evoked coughing in anesthetized guinea pigs. *Am J Physiol Regul Integr Comp Physiol* 2006;291(2):R454–463. [PubMed: 16914432]
- Canning BJ, Mazzone SB, Meeker SN, Mori N, Reynolds SM, Udem BJ. Identification of the tracheal and laryngeal afferent neurones mediating cough in anaesthetized guinea-pigs. *J Physiol* 2004;557 (Pt 2):543–558. [PubMed: 15004208]
- Canning BJ, Reynolds SM, Anukwu LU, Kajekar R, Myers AC. Endogenous neurokinins facilitate synaptic transmission in guinea pig airway parasympathetic ganglia. *Am J Physiol Regul Integr Comp Physiol* 2002;283(2):R320–330. [PubMed: 12121843]
- Chiang CH, Gabella G. Quantitative study of the ganglion neurons of the mouse trachea. *Cell Tissue Res* 1986;246(2):243–252. [PubMed: 3779806]
- Choi HK, Finkbeiner WE, Widdicombe JH. A comparative study of mammalian tracheal mucous glands. *J Anat* 2000;197(Pt 3):361–372. [PubMed: 11117623]
- Choi JY, Ianowski JP, Hanrahan JW, Wine JJ. Mucus secretion by mouse airway submucosal glands. *Pediatric Pulmonology* 2006;(Supplement 29):238.

- Choi JY, Joo NS, Wu JV, Krouse ME, Wine JJ. Human submucosal gland secretion: synergic stimulation by low levels of vip and carbachol is lost in cystic fibrosis. *Pediatric Pulmonology* 2006;(Supplement 29):238.
- Choi JY, Joo NS, Wu JV, Krouse ME, Wine JJ. Submucosal gland secretion in pig airways: synergistic stimulation by low levels of vip and carbachol. *Pediatric Pulmonology* 2006;(Supplement 29):238.
- Coburn RF. The anatomy of the ferret paratracheal parasympathetic nerve-ganglion plexus. *Exp Lung Res* 1984;7(1):1–9. [PubMed: 6510374]
- Coburn RF, Kalia MP. Morphological features of spiking and nonspiking cells in the paratracheal ganglion of the ferret. *J Comp Neurol* 1986;254(3):341–351. [PubMed: 3794010]
- Cole AM, Waring AJ. The role of defensins in lung biology and therapy. *Am J Respir Med* 2002;1(4):249–259. [PubMed: 14720045]
- Coleridge HM, Coleridge JC. Pulmonary reflexes: neural mechanisms of pulmonary defense. *Annu Rev Physiol* 1994;56:69–91. [PubMed: 8010756]
- Coles SJ, Neill KH, Reid LM. Potent stimulation of glycoprotein secretion in canine trachea by substance P. *J Appl Physiol* 1984;57(5):1323–1327. [PubMed: 6084001]
- Crews A, Taylor AE, Ballard ST. Liquid transport properties of porcine tracheal epithelium. *J Appl Physiol* 2001;91(2):797–802. [PubMed: 11457796]
- Dajani R, Zhang Y, Taft PJ, Travis SM, Starner TD, Olsen A, Zabner J, Welsh MJ, Engelhardt JF. Lysozyme secretion by submucosal glands protects the airway from bacterial infection. *Am J Respir Cell Mol Biol* 2005;32(6):548–552. [PubMed: 15746432]
- Davis B, Chinn R, Gold J, Popovac D, Widdicombe JG, Nadel JA. Hypoxemia reflexly increases secretion from tracheal submucosal glands in dogs. *J Appl Physiol* 1982;52(6):1416–1419. [PubMed: 7107454]
- Davis B, Roberts AM, Coleridge HM, Coleridge JC. Reflex tracheal gland secretion evoked by stimulation of bronchial C- fibers in dogs. *J Appl Physiol* 1982;53(4):985–991. [PubMed: 7153131]
- Davis B, Tseng HC. Neural regulation of lysozyme secretion from tracheal submucosal glands of ferrets in vivo. *J Appl Physiol* 1991;71(3):939–944. [PubMed: 1721905]
- Devor DC, Singh AK, Lambert LC, DeLuca A, Frizzell RA, Bridges RJ. Bicarbonate and chloride secretion in Calu-3 human airway epithelial cells. *J Gen Physiol* 1999;113(5):743–760. [PubMed: 10228185]
- Dey RD. Controlling from within: neurophysiological plasticity of parasympathetic airway neurons. *Am J Physiol Lung Cell Mol Physiol* 2003;284(4):L578–580. [PubMed: 12618419]
- Dey RD, Altemus JB, Michalkiewicz M. Distribution of vasoactive intestinal peptide- and substance P-containing nerves originating from neurons of airway ganglia in cat bronchi. *J Comp Neurol* 1991;304(2):330–340. [PubMed: 1707900]
- Dey RD, Altemus JB, Rodd A, Mayer B, Said SI, Coburn RF. Neurochemical characterization of intrinsic neurons in ferret tracheal plexus. *Am J Respir Cell Mol Biol* 1996;14(3):207–216. [PubMed: 8845170]
- Dey RD, Hoffpauir J, Said SI. Co-localization of vasoactive intestinal peptide- and substance P-containing nerves in cat bronchi. *Neuroscience* 1988;24(1):275–281. [PubMed: 2452994]
- Dey RD, Mayer B, Said SI. Colocalization of vasoactive intestinal peptide and nitric oxide synthase in neurons of the ferret trachea. *Neuroscience* 1993;54(4):839–843. [PubMed: 7688104]
- Dey RD, Satterfield B, Altemus JB. Innervation of tracheal epithelium and smooth muscle by neurons in airway ganglia. *Anat Rec* 1999;254(2):166–172. [PubMed: 9972801]
- Dey RD, Shannon WA Jr, Said SI. Localization of VIP-immunoreactive nerves in airways and pulmonary vessels of dogs, cat, and human subjects. *Cell Tissue Res* 1981;220(2):231–238. [PubMed: 7296630]
- Dey RD, Zhu W. Origin of galanin in nerves of cat airways and colocalization with vasoactive intestinal peptide. *Cell Tissue Res* 1993;273(1):193–200. [PubMed: 7689936]
- Duszyk M. CFTR and lysozyme secretion in human airway epithelial cells. *Pflugers Arch* 2001;443(Suppl 1):S45–49. [PubMed: 11845302]
- Elkins MR, Robinson M, Rose BR, Harbour C, Moriarty CP, Marks GB, Belousova EG, Xuan W, Bye PT. A controlled trial of long-term inhaled hypertonic saline in patients with cystic fibrosis. *N Engl J Med* 2006;354(3):229–240. [PubMed: 16421364]

- Emmelin N, Garrett JR, Gjorstrup P. Supporting effects of myoepithelial cells in submandibular glands of dogs when acting against increased intraluminal pressure. *J Physiol* 1977;268(1):73–85. [PubMed: 874910]
- Finkbeiner WE, Carrier SD, Teresi CE. Reverse transcription-polymerase chain reaction (RT-PCR) phenotypic analysis of cell cultures of human tracheal epithelium, tracheobronchial glands, and lung carcinomas. *Am J Respir Cell Mol Biol* 1993;9(5):547–556. [PubMed: 7692897]
- Fischer A, Canning BJ, Kummer W. Correlation of vasoactive intestinal peptide and nitric oxide synthase with choline acetyltransferase in the airway innervation. *Ann N Y Acad Sci* 1996;805:717–722. [PubMed: 8993467]
- Fischer A, Canning BJ, Undem BJ, Kummer W. Evidence for an esophageal origin of VIP-IR and NO synthase-IR nerves innervating the guinea pig trachealis: a retrograde neuronal tracing and immunohistochemical analysis. *J Comp Neurol* 1998;394(3):326–334. [PubMed: 9579396]
- Fischer A, Hoffmann B. Nitric oxide synthase in neurons and nerve fibers of lower airways and in vagal sensory ganglia of man. Correlation with neuropeptides. *Am J Respir Crit Care Med* 1996;154(1):209–216. [PubMed: 8680682]
- Fisher AW. The Intrinsic Innervation of the Trachea. *J Anat* 1964;98:117–124. [PubMed: 14109806]
- Fontan JJ, Cortright DN, Krause JE, Velloff CR, Karpitskyi VV, Carver TW Jr, Shapiro SD, Mora BN. Substance P and neurokinin-1 receptor expression by intrinsic airway neurons in the rat. *Am J Physiol Lung Cell Mol Physiol* 2000;278(2):L344–355. [PubMed: 10666119]
- Ganz T. Antimicrobial polypeptides. *J Leukoc Biol* 2004;75(1):34–38. [PubMed: 12960278]
- Gashi AA, Borson DB, Finkbeiner WE, Nadel JA, Basbaum CB. Neuropeptides degranulate serous cells of ferret tracheal glands. *Am J Physiol* 1986;251(2 Pt 1):C223–229. [PubMed: 2426958]
- German VF, Corrales R, Ueki IF, Nadel JA. Reflex stimulation of tracheal mucus gland secretion by gastric irritation in cats. *J Appl Physiol* 1982;52(5):1153–1155. [PubMed: 7096138]
- German VF, Ueki IF, Nadel JA. Micropipette measurement of airway submucosal gland secretion: laryngeal reflex. *Am Rev Respir Dis* 1980;122(3):413–416. [PubMed: 7416616]
- Gerson C, Sabater J, Scuri M, Torbati A, Coffey R, Abraham JW, Lauredo I, Forteza R, Wanner A, Salathe M, Abraham WM, Conner GE. The lactoperoxidase system functions in bacterial clearance of airways. *Am J Respir Cell Mol Biol* 2000;22(6):665–671. [PubMed: 10837362]
- Goetz DH, Holmes MA, Borregaard N, Bluhm ME, Raymond KN, Strong RK. The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. *Mol Cell* 2002;10(5):1033–1043. [PubMed: 12453412]
- Haws C, Finkbeiner WE, Widdicombe JH, Wine JJ. CFTR in Calu-3 human airway cells: channel properties and role in cAMP-activated Cl⁻ conductance. *Am J Physiol* 1994;266(5 Pt 1):L502–512. [PubMed: 7515579]
- Haxhiu MA, Chavez JC, Pichiule P, Erokwu B, Dreshaj IA. The excitatory amino acid glutamate mediates reflexly increased tracheal blood flow and airway submucosal gland secretion. *Brain Res* 2000;883(1):77–86. [PubMed: 11063990]
- Haxhiu MA, Cherniack NS, Strohl KP. Reflex responses of laryngeal and pharyngeal submucosal glands in dogs. *J Appl Physiol* 1991;71(5):1669–1673. [PubMed: 1722199]
- Haxhiu MA, Haxhiu-Poskurica B, Moracic V, Carlo WA, Martin RJ. Reflex and chemical responses of tracheal submucosal glands in piglets. *Respir Physiol* 1990;82(3):267–277. [PubMed: 1706884]
- Haxhiu MA, Kc P, Moore CT, Acquah SS, Wilson CG, Zaidi SI, Massari VJ, Ferguson DG. Brain stem excitatory and inhibitory signaling pathways regulating bronchoconstrictive responses. *J Appl Physiol* 2005;98(6):1961–1982. [PubMed: 15894534]
- Haxhiu MA, van Lunteren E, Cherniack NS. Central effects of tachykinin peptide on tracheal secretion. *Respir Physiol* 1991;86(3):405–414. [PubMed: 1724094]
- Haxhiu MA, Van Lunteren E, Cherniack NS. Influence of ventrolateral surface of medulla on tracheal gland secretion. *J Appl Physiol* 1991;71(5):1663–1668. [PubMed: 1761461]
- Hejal R, Strohl KP, Erokwu B, Cherniack NS, Haxhiu MA. Pathways and mechanisms involved in neural control of laryngeal submucosal gland secretion. *J Appl Physiol* 1993;75(6):2347–2352. [PubMed: 8125850]
- Hejal R, Strohl KP, Erokwu B, Cherniack NS, Haxhiu MA. Effect of hypoxia on reflex responses of tracheal submucosal glands. *J Appl Physiol* 1995;78(5):1651–1656. [PubMed: 7544338]

- Hunter DD, Undem BJ. Identification and substance P content of vagal afferent neurons innervating the epithelium of the guinea pig trachea. *Am J Respir Crit Care Med* 1999;159(6):1943–1948. [PubMed: 10351943]
- Hutchings HA, Morris S, Eccles R, Jawad MS. Voluntary suppression of cough induced by inhalation of capsaicin in healthy volunteers. *Respir Med* 1993;87(5):379–382. [PubMed: 8209058]
- Ianowski JP, Choi JY, Wine JJ, Hanrahan JW. Mucus secretion by CFTR $-/-$ mouse airway submucosal glands. *Pediatric Pulmonology* 2006;(Supplement 29):264.
- Ianowski JP, Choi JY, Wine JJ, Hanrahan JW. Mucus secretion by CFTR $-/-$ mouse airway submucosal glands. *J Physiol*. 2007in press
- Inglis SK, Corboz MR, Ballard ST. Effect of anion secretion inhibitors on mucin content of airway submucosal gland ducts. *Am J Physiol* 1998;274(5 Pt 1):L762–766. [PubMed: 9612291]
- Inglis SK, Corboz MR, Taylor AE, Ballard ST. Effect of anion transport inhibition on mucus secretion by airway submucosal glands. *Am J Physiol* 1997;272(2 Pt 1):L372–377. [PubMed: 9124389]
- Inglis SK, Corboz MR, Taylor AE, Ballard ST. In situ visualization of bronchial submucosal glands and their secretory response to acetylcholine. *Am J Physiol* 1997;272(2 Pt 1):L203–210. [PubMed: 9124370]
- Irokawa T, Krouse ME, Joo NS, Wu JV, Wine JJ. A “virtual gland” method for quantifying epithelial fluid secretion. *Am J Physiol Lung Cell Mol Physiol* 2004;287(4):L784–793. [PubMed: 15169677]
- Jayaraman S, Joo NS, Reitz B, Wine JJ, Verkman AS. Submucosal gland secretions in airways from cystic fibrosis patients have normal $[Na^+]$ and pH but elevated viscosity. *Proc Natl Acad Sci U S A* 2001;98(14):8119–8123. [PubMed: 11427704]
- Joo NS, Irokawa T, Robbins RC, Wine JJ. Hyposecretion, not hyperabsorption, is the basic defect of cystic fibrosis airway glands. *J Biol Chem* 2006;281(11):7392–7398. [PubMed: 16410244]
- Joo NS, Irokawa T, Wu JV, Robbins RC, Whyte RI, Wine JJ. Absent Secretion to Vasoactive Intestinal Peptide in Cystic Fibrosis Airway Glands. *J Biol Chem* 2002;277(52):50710–50715. [PubMed: 12368280]
- Joo NS, Krouse ME, Wu JV, Saenz Y, Jayaraman S, Verkman AS, Wine JJ. HCO_3^- Transport in Relation to Mucus Secretion from Submucosal Glands. *Jop* 2001;2(4 Suppl):280–284. [PubMed: 11875272]
- Joo NS, Lee DJ, Wings KM, Rustagi A, Wine JJ. Regulation of antiprotease and antimicrobial protein secretion by airway submucosal gland serous cells. *J Biol Chem* 2004;279(37):38854–38860. [PubMed: 15234967]
- Joo NS, Saenz Y, Krouse ME, Wine JJ. Mucus Secretion from Single Submucosal Glands of Pig. Stimulation by Carbachol and Vasoactive Intestinal Peptide. *J Biol Chem* 2002;277(31):28167–28175. [PubMed: 12011087]
- Joo NS, Wu JV, Krouse ME, Saenz Y, Wine JJ. Optical method for quantifying rates of mucus secretion from single submucosal glands. *Am J Physiol Lung Cell Mol Physiol* 2001;281(2):L458–468. [PubMed: 11435221]
- Kajekar R, Rohde HK, Myers AC. The integrative membrane properties of human bronchial parasympathetic Ganglia neurons. *Am J Respir Crit Care Med* 2001;164(10 Pt 1):1927–1932. [PubMed: 11734448]
- Kajekar R, Undem BJ, Myers AC. Role of cyclooxygenase activation and prostaglandins in antigen-induced excitability changes of bronchial parasympathetic ganglia neurons. *Am J Physiol Lung Cell Mol Physiol* 2003;284(4):L581–587. [PubMed: 12618420]
- Kc P, Mayer CA, Haxhiu MA. Chemical profile of vagal preganglionic motor cells innervating the airways in ferrets: the absence of noncholinergic neurons. *J Appl Physiol* 2004;97(4):1508–1517. [PubMed: 15358755]
- Khakh BS, North RA. P2X receptors as cell-surface ATP sensors in health and disease. *Nature* 2006;442(7102):527–532. [PubMed: 16885977]
- Kim JS, Okamoto K, Arima S, Rubin BK. Vasoactive intestinal peptide stimulates mucus secretion, but nitric oxide has no effect on mucus secretion in the ferret trachea. *J Appl Physiol*. 2006
- Kirchgessner AL, Liu MT, Gershon MD. In situ identification and visualization of neurons that mediate enteric and enteropancreatic reflexes. *J Comp Neurol* 1996;371(2):270–286. [PubMed: 8835732]

- Knowles MR, Boucher RC. Mucus clearance as a primary innate defense mechanism for mammalian airways. *J Clin Invest* 2002;109(5):571–577. [PubMed: 11877463]
- Kubin L, Alheid GF, Zuperku EJ, McCrimmon DR. Central pathways of pulmonary and lower airway vagal afferents. *J Appl Physiol* 2006;101(2):618–627. [PubMed: 16645192]
- Kuder T, Szczurkowski A, Kuchinka J, Nowak E. The AChE-positive ganglia in the trachea and bronchi of the cat. *Folia Morphol (Warsz)* 2003;62(2):99–106. [PubMed: 12866668]
- Kusindarta DL, Atoji Y, Yamamoto Y. Nerve plexuses in the trachea and extrapulmonary bronchi of the rat. *Arch Histol Cytol* 2004;67(1):41–55. [PubMed: 15125022]
- Laitinen A. Ultrastructural organisation of intraepithelial nerves in the human airway tract. *Thorax* 1985;40(7):488–492. [PubMed: 4035614]
- Lamb D, Reid L. The tracheobronchial submucosal glands in cystic fibrosis: a qualitative and quantitative histochemical study. *Br J Dis Chest* 1972;66(4):239–247. [PubMed: 5083431]
- Landois, L. *A Textbook of Human Physiology*. London: Charles Griffin; 1885.
- Lee MC, Penland CM, Widdicombe JH, Wine JJ. Evidence that Calu-3 human airway cells secrete bicarbonate. *Am J Physiol* 1998;274(3 Pt 1):L450–453. [PubMed: 9530182]
- Levin MH, Sullivan S, Nielson D, Yang B, Finkbeiner WE, Verkman AS. Hypertonic saline therapy in cystic fibrosis: Evidence against the proposed mechanism involving aquaporins. *J Biol Chem* 2006;281(35):25803–25812. [PubMed: 16829520]
- Lundberg JM, Hokfelt T, Martling CR, Saria A, Cuello C. Substance P-immunoreactive sensory nerves in the lower respiratory tract of various mammals including man. *Cell Tissue Res* 1984;235(2):251–261. [PubMed: 6200231]
- Mazzone SB, Canning BJ. Central nervous system control of the airways: pharmacological implications. *Curr Opin Pharmacol* 2002;2(3):220–228. [PubMed: 12020461]
- Meyrick B, Reid L. Ultrastructure of cells in the human bronchial submucosal glands. *J Anat* 1970;107(2):281–299. [PubMed: 5487122]
- Meyrick B, Sturgess JM, Reid L. A reconstruction of the duct system and secretory tubules of the human bronchial submucosal gland. *Thorax* 1969;24(6):729–736. [PubMed: 5350723]
- Mitchell RA, Herbert DA, Baker DG, Basbaum CB. In vivo activity of tracheal parasympathetic ganglion cells innervating tracheal smooth muscle. *Brain Res* 1987;437(1):157–160. [PubMed: 2827863]
- Myers A, Undem B, Kummer W. Anatomical and electrophysiological comparison of the sensory innervation of bronchial and tracheal parasympathetic ganglion neurons. *J Auton Nerv Syst* 1996;61(2):162–168. [PubMed: 8946336]
- Myers AC. Anatomical characteristics of tonic and phasic postganglionic neurons in guinea pig bronchial parasympathetic ganglia. *J Comp Neurol* 2000;419(4):439–450. [PubMed: 10742714]
- Myers AC. Transmission in autonomic ganglia. *Respir Physiol* 2001;125(12):99–111. [PubMed: 11240155]
- Myers AC, Goldie RG, Hay DW. A novel role for tachykinin neurokinin-3 receptors in regulation of human bronchial Ganglia neurons. *Am J Respir Crit Care Med* 2005;171(3):212–216. [PubMed: 15477495]
- Myers AC, Undem BJ. Electrophysiological effects of tachykinins and capsaicin on guinea-pig bronchial parasympathetic ganglion neurones. *J Physiol* 1993;470:665–679. [PubMed: 7508508]
- Myers AC, Undem BJ. Antigen depolarizes guinea pig bronchial parasympathetic ganglion neurons by activation of histamine H1 receptors. *Am J Physiol* 1995;268(6 Pt 1):L879–884. [PubMed: 7611429]
- Myers AC, Undem BJ. Muscarinic receptor regulation of synaptic transmission in airway parasympathetic ganglia. *Am J Physiol* 1996;270(4 Pt 1):L630–636. [PubMed: 8928823]
- Nadel JA, Davis B, Phipps RJ. Control of mucus secretion and ion transport in airways. *Annu Rev Physiol* 1979;41:369–381. [PubMed: 373597]
- Newhouse M, Sanchis J, Bienenstock J. Lung defense mechanisms (first of two parts). *N Engl J Med* 1976;295(18):990–998. [PubMed: 787790]
- Oh EJ, Mazzone SB, Canning BJ, Weinreich D. Reflex regulation of airway sympathetic nerves in guinea-pigs. *J Physiol* 2006;573(Pt 1):549–564. [PubMed: 16581869]

- Pastor LM, Ferran A, Calvo A, Sprekelsen C, Horn R, Marin JA. Morphological and histochemical study of human submucosal laryngeal glands. *Anat Rec* 1994;239(4):453–467. [PubMed: 7978368]
- Peatfield AC, Barnes PJ, Bratcher C, Nadel JA, Davis B. Vasoactive intestinal peptide stimulates tracheal submucosal gland secretion in ferret. *Am Rev Respir Dis* 1983;128(1):89–93. [PubMed: 6870072]
- Pedersen KE, Meeker SN, Riccio MM, Udem BJ. Selective stimulation of jugular ganglion afferent neurons in guinea pig airways by hypertonic saline. *J Appl Physiol* 1998;84(2):499–506. [PubMed: 9475859]
- Perez Fontan JJ, Velloff CR. Labeling of vagal motoneurons and central afferents after injection of cholera toxin B into the airway lumen. *Am J Physiol Lung Cell Mol Physiol* 2001;280(1):L152–164. [PubMed: 11133505]
- Petersen OH, Sutton R. Ca²⁺ signalling and pancreatitis: effects of alcohol, bile and coffee. *Trends Pharmacol Sci* 2006;27(2):113–120. [PubMed: 16406087]
- Phillips JE, Hey JA, Corboz MR. Computer assisted measurement of airway gland secretions by the hillocks technique. *Comput Methods Programs Biomed* 2002;68(3):215–222. [PubMed: 12074848]
- Phillips JE, Hey JA, Corboz MR. Effects of ion transport inhibitors on MCh-mediated secretion from porcine airway submucosal glands. *J Appl Physiol* 2002;93(3):873–881. [PubMed: 12183480]
- Phillips JE, Hey JA, Corboz MR. Tachykinin NK3 and NK1 receptor activation elicits secretion from porcine airway submucosal glands. *Br J Pharmacol* 2003;138(1):254–260. [PubMed: 12522097]
- Pisarrri TE, Coleridge HM, Coleridge JC. Reflex bronchial vasodilation in dogs evoked by injection of a small volume of water into a bronchus. *J Appl Physiol* 1993;75(5):2195–2202. [PubMed: 8307879]
- Pisarrri TE, Jonzon A, Coleridge HM, Coleridge JC. Vagal afferent and reflex responses to changes in surface osmolarity in lower airways of dogs. *J Appl Physiol* 1992;73(6):2305–2313. [PubMed: 1490937]
- Quinton PM. Composition and control of secretions from tracheal bronchial submucosal glands. *Nature* 1979;279(5713):551–552. [PubMed: 450103]
- Robinson M, Regnis JA, Bailey DL, King M, Bautovich GJ, Bye PT. Effect of hypertonic saline, amiloride, and cough on mucociliary clearance in patients with cystic fibrosis. *Am J Respir Crit Care Med* 1996;153(5):1503–1509. [PubMed: 8630593]
- Salinas D, Haggie PM, Thiagarajah JR, Song Y, Rosbe K, Finkbeiner WE, Nielson DW, Verkman AS. Submucosal gland dysfunction as a primary defect in cystic fibrosis. *Faseb J* 2005;19(3):431–433. [PubMed: 15596485]
- Schultz HD, Davis B, Coleridge HM, Coleridge JC. Cigarette smoke in lungs evokes reflex increase in tracheal submucosal gland secretion in dogs. *J Appl Physiol* 1991;71(3):900–909. [PubMed: 1757327]
- Schultz HD, Roberts AM, Bratcher C, Coleridge HM, Coleridge JC, Davis B. Pulmonary C-fibers reflexly increase secretion by tracheal submucosal glands in dogs. *J Appl Physiol* 1985;58(3):907–910. [PubMed: 3980392]
- Shen BQ, Finkbeiner WE, Wine JJ, Mrsny RJ, Widdicombe JH. Calu-3: a human airway epithelial cell line that shows cAMP-dependent Cl⁻ secretion. *Am J Physiol* 1994;266(5 Pt 1):L493–501. [PubMed: 7515578]
- Shimura S, Sasaki T, Ikeda K, Sasaki H, Takishima T. VIP augments cholinergic-induced glycoconjugate secretion in tracheal submucosal glands. *J Appl Physiol* 1988;65(6):2537–2544. [PubMed: 2851006]
- Shimura S, Sasaki T, Okayama H, Sasaki H, Takishima T. Effect of substance P on mucus secretion of isolated submucosal gland from feline trachea. *J Appl Physiol* 1987;63(2):646–653. [PubMed: 2443476]
- Song Y, Salinas D, Nielson DW, Verkman AS. Hyperacidity of secreted fluid from submucosal glands in early cystic fibrosis. *Am J Physiol Cell Physiol* 2006;290(3):C741–749. [PubMed: 16207791]
- Thiagarajah JR, Song Y, Haggie PM, Verkman AS. A small molecule CFTR inhibitor produces cystic fibrosis-like submucosal gland fluid secretions in normal airways. *Faseb J* 2004;18(7):875–877. [PubMed: 15001557]
- Tos M. Development of the tracheal glands in man. Number, density, structure, shape, and distribution of mucous glands elucidated by quantitative studies of whole mounts. *Acta Pathol Microbiol Scand* 1966;68(Suppl 185):1–130. [PubMed: 5954971]

- Trout L, Corboz MR, Ballard ST. Mechanism of substance P-induced liquid secretion across bronchial epithelium. *Am J Physiol Lung Cell Mol Physiol* 2001;281(3):L639–645. [PubMed: 11504691]
- Trout L, Gatzky JT, Ballard ST. Acetylcholine-induced liquid secretion by bronchial epithelium: role of Cl⁻ and HCO₃⁻ transport. *Am J Physiol* 1998;275(6 Pt 1):L1095–1099. [PubMed: 9843846]
- Trout L, King M, Feng W, Inglis SK, Ballard ST. Inhibition of airway liquid secretion and its effect on the physical properties of airway mucus. *Am J Physiol* 1998;274(2 Pt 1):L258–263. [PubMed: 9486211]
- Trout L, Townsley MI, Bowden AL, Ballard ST. Disruptive effects of anion secretion inhibitors on airway mucus morphology in isolated perfused pig lung. *J Physiol* 2003;549(Pt 3):845–853. [PubMed: 12702745]
- Uddman R, Alumets J, Densert O, Hakanson R, Sundler F. Occurrence and distribution of VIP nerves in the nasal mucosa and tracheobronchial wall. *Acta Otolaryngol* 1978;86(56):443–448. [PubMed: 362800]
- Udem BJ, Chuaychoo B, Lee MG, Weinreich D, Myers AC, Kollarik M. Subtypes of vagal afferent C-fibres in guinea-pig lungs. *J Physiol* 2004;556(Pt 3):905–917. [PubMed: 14978204]
- Van Genechten J, Brouns I, Scheuermann DW, Timmermans JP, Adriaensen D. Reduced number of intrinsic pulmonary nitroergic neurons in Fawn-Hooded rats as compared to control rat strains. *Anat Rec A Discov Mol Cell Evol Biol* 2003;272(1):446–453. [PubMed: 12704702]
- Verkman AS, Song Y, Thiagarajah JR. Role of airway surface liquid and submucosal glands in cystic fibrosis lung disease. *Am J Physiol Cell Physiol* 2003;284(1):C2–15. [PubMed: 12475759]
- Wagner U, Fehmann HC, Bredenkroter D, Yu F, Barth PJ, von Wichert P. Galanin and somatostatin inhibition of substance P-induced airway mucus secretion in the rat. *Neuropeptides* 1995;28(1):59–64. [PubMed: 7538203]
- Weichselbaum M, Sparrow MP, Hamilton EJ, Thompson PJ, Knight DA. A confocal microscopic study of solitary pulmonary neuroendocrine cells in human airway epithelium. *Respir Res* 2005;6:115. [PubMed: 16216130]
- Widdicombe JG. Action potentials in parasympathetic and sympathetic efferent fibres to the trachea and lungs of dogs and cats. *J Physiol* 1966;186(1):56–88. [PubMed: 5914258]
- Widdicombe, JG. Defensive mechanisms of the respiratory system. In: Widdicombe, JG., editor. *International Review of Physiology: Respiratory Physiology II*. 14. Baltimore: Univ. Park Press; 1977. p. 291–315.
- Widdicombe JH, Chen LL, Sporer H, Choi HK, Pecson IS, Bastacky SJ. Distribution of tracheal and laryngeal mucous glands in some rodents and the rabbit. *J Anat* 2001;198(Pt 2):207–221. [PubMed: 11273045]
- Widdicombe JH, Pecson IS. Distribution and numbers of mucous glands in the horse trachea. *Equine Vet J* 2002;34(6):630–633. [PubMed: 12358006]
- Worlitzsch D, Tarran R, Ulrich M, Schwab U, Cekici A, Meyer KC, Birrer P, Bellon G, Berger J, Weiss T, Botzenhart K, Yankaskas JR, Randell S, Boucher RC, Doring G. Effects of reduced mucus oxygen concentration in airway *Pseudomonas* infections of cystic fibrosis patients. *J Clin Invest* 2002;109(3):317–325. [PubMed: 11827991]
- Wu ZX, Dey RD. Nerve growth factor-enhanced airway responsiveness involves substance P in ferret intrinsic airway neurons. *Am J Physiol Lung Cell Mol Physiol* 2006;291(1):L111–118. [PubMed: 16461428]
- Wu ZX, Maize DF Jr, Satterfield BE, Frazer DG, Fedan JS, Dey RD. Role of intrinsic airway neurons in ozone-induced airway hyperresponsiveness in ferret trachea. *J Appl Physiol* 2001;91(1):371–378. [PubMed: 11408454]
- Yamamoto Y, Ootsuka T, Atoji Y, Suzuki Y. Morphological and quantitative study of the intrinsic nerve plexuses of the canine trachea as revealed by immunohistochemical staining of protein gene product 9.5. *Anat Rec* 1998;250(4):438–447. [PubMed: 9566534]
- Yu J, Schultz HD, Goodman J, Coleridge JC, Coleridge HM, Davis B. Pulmonary rapidly adapting receptors reflexly increase airway secretion in dogs. *J Appl Physiol* 1989;67(2):682–687. [PubMed: 2793670]
- Zhu W, Dey RD. Projections and pathways of VIP- and nNOS-containing airway neurons in ferret trachea. *Am J Respir Cell Mol Biol* 2001;24(1):38–43. [PubMed: 11152648]

Fig 1.

The Airway submucosal glands. A. Secreted mucus from human glands, visualized using the oil-layer method for optical measurement of secretion rates. B. Glands in a fetal human airway. PAS-stained whole mount of a section of trachea reveals some of the glands. C. Living human submucosal gland. A. from (Joo, Irokawa et al. 2002); B. from (Tos 1966); C. and D. Wine, unpublished observations.

Fig 2.

Overview of airway innervation by the ANS. The parasympathetic system is shown with solid lines to the left; the sympathetic system is shown with dashed lines to the right. In humans, sympathetic innervation appears to have minor effects and will be ignored in this review. Vagal afferents arise from cell bodies in the jugular/nodose ganglia and send their terminals into the nucleus of the solitary tract in the brainstem. Interneurons then transmit the information to the airway vagal preganglionic neurons (AVPNs) in the nucleus of the vagus and the nucleus ambiguus. These descend to excite neurons in the airway intrinsic nervous system. Modified from Oh, *J Physiol*, 2006, **573**, 549 B.

Fig 3. Nerve plexuses in the ferret trachea visualized with stain for acetylcholinesterase activity. 1. Montage tracing of dorsal view of nerves and ganglia in newborn ferret trachea from larynx (top) to the carina. Vagus nerves (V), recurrent laryngeal nerves (R), para-recurrent laryngeal nerves (P), longitudinal nerve trunks (LGT). 2-3. Photomicrographs of the dorsal and right lateral exterior surfaces at rostral, middle and caudal regions of the trachea. From (Baker, McDonald et al. 1986).

Fig 4.

The intrinsic neural network of the dog trachea has neurons in 4 different layers (the intramuscular plexus lacks nerve cell bodies). A. Schematic of dog intrinsic nervous system. B. PGP 9.5-reactive neurons in the submucosal plexus. Arrow points to a small ganglion in this deep layer, arrow heads point to neural processes. A submucosal gland is shown to the right. From (Yamamoto, Ootsuka et al. 1998)

Fig 5.

Intrinsic airway neurons have complex morphologies. A. An intrinsic airway neuron from the longitudinal tract of the ferret showing axon running through an adjacent ganglion and then projecting to the trachealis muscle; another process may go to ganglia of the superficial muscle plexus. B. Overview of a portion of the ferret intrinsic nervous system. C, D. Images of airway intrinsic ganglia from human trachea; E. filled human airway intrinsic neuron (asterisk indicates axon). A from ref (Coburn and Kalia 1986). B. from ref (Baker, McDonald et al. 1986) D-F from ref (Kajekar, Rohde et al. 2001).

Fig 6.

Electrophysiology of intrinsic airway neurons. A-B. Rhythmic, breathing related firing patterns of cat airway intrinsic neurons. Vagal Postganglionic Neurons in cat trachea fire in synchrony with the respiratory rhythm. (A) Large units (61 μm av.) fired during inspiration and had axons to trachealis muscle. (B) Small units (36 μm av.) that fired during expiration were more lateral and near intercartilage spaces, their axons coursed toward the glands. Phrenic n. activity is shown on bottom trace. C-D. Spontaneous EPSPs in rat intrinsic airway neurons. C. EPSPs in a tonic-firing neurone. Synaptic activity was greatly reduced by 330 nm TTX and abolished by 100 μM hexamethonium. D. Another cell with spontaneous bursts of six EPSPs repeated every 3-5 sec. D₁, at -52 mV resting membrane potential the cell only fired in response to the largest EPSPs. With depolarized current injection, spike discharge increased (D₂). Hyperpolarization inhibited spike discharge and increased EPSP amplitudes (D₃). Inset shows EPSPs at increased temporal resolution. (Action potentials were attenuated by the pen recorder.) E-F. *Fast excitatory post-synaptic potentials in human bronchial intrinsic neurons.* (E) Responses of a bronchial ganglia neuron to 10 consecutive peribronchial nerve stimulations (shock artifact at vertical arrow; 1.0 ms, 20 V, 0.5 Hz). Fast EPSPs were subthreshold for action potential generation and graded in amplitude. (F) A single stimulus (shock artifact at arrow) to the preganglionic nerve trunk elicited three temporally distinct EPSPs (arrowheads) indicating convergence of preganglionic axons. Dashed line is resting membrane potential of -51 mV. A, B from ref. (Mitchell, Herbert et al. 1987) Reprinted with permission from the Elsevier; C, D from ref. (Allen and Burnstock 1990), E, F. (Kajekar, Rohde et al. 2001).

Fig 7.

Neural connections among cholinergic, longitudinal tract neurons and VIP-, nNOS-expressing neurons of the superficial muscular plexus. A. Labeling of neuronal cell bodies in a noninjected longitudinal tract ganglion (LT) (A) and a superficial muscle plexus ganglion (B) after biotinylated dextran-amine (BDA) injection in an adjacent LT ganglion (not shown). (A) Montaged micrograph of an LT ganglion distant from the injection site. Anterogradely labeled fibers terminate around unfilled cell bodies seen as basketlike complexes with associated punctate terminals (arrowheads). Labeling in cell bodies of LT ganglion (asterisks) indicate retrograde filling. Labeled axons course through the ganglion (arrows). B. BDA-labeled terminals form a basketlike network around an unlabeled SMP nerve cell body. Individual filled axons in nerve bundles are seen (arrowheads). Scale bar: 80 mm. C. SMP ganglion after injection of combined fluororuby (FR) and BDA injection. Retrograde labeling by FR fills a nerve cell body in an SMP ganglion. The cell body is surrounded by basketlike fibers that are anterogradely labeled with BDA. D. Montage of LT and nearby SMP ganglia after BDA injection. Labeled nerve fibers course along the trunk and branch to the SMP (arrow), demonstrating projection from injected LT ganglion to the SMP ganglion. (Zhu and Dey 2001).

Fig 8.

Integrative physiological studies of the airway emergency defense reflexes. A. Experimental set up for concurrent measurement of trachealis tension, blood flow and gland secretion in vivo. B. Panels from top to bottom show gland secretion measured with the tantalum-hillocks technique, ventilation (pause in ventilation at point of maximum lung deflation was the stimulus in this experiment) and measure of blood flow. C. Schematic of vagal parasympathetic reflex pathway that is engaged in this experiment. Inset shows airway-related vagal preganglionic (AVPNs) from the nucleus ambiguus that had been backfilled by injecting label in the airway. A, B. From ref (Haxhiu, Kc et al. 2005), C from ref (Oh, Mazzone et al. 2006), inset from ref. (Kc, Mayer et al. 2004)

Fig 9.

Selective responding of airway afferents to hypertonic saline solutions according to their ganglion of origin or (inset) whether they are A δ or C fibers. (a) Responses of a neuron in the jugular ganglion to 4% NaCl, and (b) the weaker responses of a nodose neuron to increasing concentrations of NaCl. The mean responses of both kinds of neurons are shown in the main diagram; the inset shows the higher responsiveness of jugular A δ fibers vs. jugular C fibers. A δ afferents are most responsive. (Pedersen, Meeker et al. 1998), with permission of the author and the American Physiological Society.

Fig 10.

Hypothetical schema for neural control of airway glands in normal (A) and emergency conditions. A. Normally, nose breathing of ordinary air at ordinary rates produces low vagal tone. The network interactions among intrinsic airway neurons filters out much of the descending neural impulse traffic, leaving glands to be stimulated by low levels of cholinergic and non-cholinergic (largely VIP) input—this kind of secretion is dominated by serous cell secretion and utilizes CFTR. Airway sensory receptors can increase secretion to mild stimuli via local and axon reflexes. B. During acute airway stress, strong descending vagal excitation produces strong cholinergic input that stimulates high levels of cholinergic input. This recruits secretion mediated by non-CFTR mechanisms including calcium-activated chloride channels. (Gland images from (Pastor, Ferran et al. 1994)).