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Review Article

Laryngeal Chemoreflex in Health and Disease: A Review

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Abstract

The larynx plays a key role in airway protection via the laryngeal chemoreflex (LCR). This involuntary reflex can be evoked when hazardous substances activate mucosal receptors, which send signals to be processed within the brainstem. Although the LCR is meant to be protective, the reflex can become hyperstimulated, even to benign stimuli, which can result in pathological disorders, such as chronic cough and inducible laryngeal obstruction. In this review, we will outline the mechanism of the LCR and its associated pathological disorders.

Key words: airway receptors, chemoreflex, cough, larynx

Introduction

The larynx is arguably one of the most complex organs in the human body, carrying out and coordinating vital functions of breathing, swallowing, and phonation. First acquired in aquatic vertebrates almost 370 million years ago with the appearance of lungfishes that were capable of both gill and lung breathing (Negus 1955, 1957), the larynx served as a valve to prevent drowning of the airways while diving. This breath-holding adaptation, known as the dive reflex, allowed aquatic reptiles and mammals to dive and feed simultaneously (Tchobroutsky et al. 1969; Bartlett 1989). As species evolved, so did laryngeal functions (Bartlett 1989; Thach 2001; Praud 2010). Despite its vast functionality, the laryngeal role in airway protection has been evolutionarily preserved.

Adjusting to multiple functional modalities requires continuous monitoring of the mucosal environment by way of cellular and neural receptors. These receptors are fundamental components of an afferent pathway that coordinates an airway protective reflex (Bradley 2000) known as the laryngeal chemoreflex (LCR). As an evolution of the dive reflex, the LCR results in physiological changes, usually containing both parasympathetic components, such as apnea, bradycardia, and laryngospasm, and sympathetic components, such as systemic hypertension and blood flow redistribution (Thach 2001). In mammals, the LCR is mainly present in newborns and infants, with even more robust and prolonged responses occurring in fetuses and premature infants (Downing and Lee 1975; Jadcherla et al. 2006; Reix et al. 2007; Praud 2010). The reflex is thought to be largely a feto-protective reflex present to not only prevent aspiration during birth but also during the immediate postnatal development period. Prior to birth, the airways are bathed in a hyperchloremic pulmonary mucus (170 mEq/L) secreted by the developing pulmonary epithelium, whereas the fetus itself is surrounded by hypochloremic amniotic fluid (80 mEq/L; Thach 2001). It has been suggested that this in-utero chloride differential sets the laryngeal receptor threshold, thereby preventing aspiration of amniotic fluid during birth with apnea and glottic closure. In the postnatal period, laryngeal receptors continue to be sensitive to hypochloremic solutions (Thach 2001). With age, maturation of neural circuitry within the brainstem allows for progression of the primitive LCR from prolonged apnea and glottic closure to a cough reflex (Thach 2001), which also serves to protect the airways by expelling unwanted substances. Abnormalities affecting this circuit, either at the level of the mucosal afferent receptors, within the brainstem, or efferent signals, can contribute to certain pathological disorders by reactivation of the primitive

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LCR. For example, abnormalities surrounding the laryngeal mucosa can result in physiologic manifestations similar to the LCR by heightening the sensitivity of the reflex, including chronic cough (Canning et al. 2004), dysphagia (Ding et al. 2013), prolonged apnea (Thach 2001; Praud 2010), and perhaps the most severe sudden infant death syndrome (Downing and Lee 1975; Page and Jeffery 2000; Leiter and Böhm 2007; Thach 2008; Praud 2010). Common laryngeal disorders including induced laryngeal obstruction (ILO; also known as paradoxical vocal fold motion disorder) and vocal hyperfunction may also be associated with the LCR (Morrison et al. 1999).

The larynx has evolved exceptional ability to respond to an extensive list of stimuli. Mucosal surveillance by way of laryngeal receptors is the first critical step in triggering the response that ultimately protects our airways. Activation of these receptors results in afferent signals sent through the laryngeal nerves to trigger the LCR.

Laryngeal receptors

Afferent laryngeal receptors are distributed throughout the larynx and relay sensory information from the larynx to the brainstem. They are the most densely located along the laryngeal aspect of the epiglottis, the aryepiglottic folds, and the arytenoid cartilages (Yoshida et al. 2000). This posterior distribution remains well preserved across species (Sant'Ambrogio and Widdicombe 2001; Widdicombe 2001), suggesting their organization within the larynx is key in relaying critical and timely sensory information about hazards entering the airway. For instance, herbivorous animals regurgitate large boluses of food as part of normal digestion and as such are at an increased risk of aspiration. When compared with their omnivorous relatives, herbivorous mammals have a higher number of posteriorly distributed laryngeal receptors, perhaps contributing to protection from aspiration during these regurgitation events (Shrestha et al. 1995).

Classification schemes of afferent airway receptors are varied and complex. They have been classified in numerous ways, including by their histology, neurophysiology, and adaptation patterns (Bradley 2000; Polverino et al. 2012), creating confusion in the literature. Histological studies in various species have resulted in at least four structural classifications including free nerve endings, taste buds, complex nerve endings (glomerular, corpuscular, and lamellar receptors), and muscle spindles (Widdicombe 2001). Studies using single fiber recordings of action potentials in response to specific stimuli have yielded additional classifications, including pressure receptors, cold receptors, drive receptors, irritant receptors, and C-fiber receptors. Laryngeal receptors have also been defined by their adaptation ability, including slowly adapting receptors and rapidly adapting receptors (Widdicombe 2001).The broadest physiochemical scheme divides these receptors into two groups based on the stimuli to which they respond: mechanoreceptors and chemoreceptors (Bradley 2000; Widdicombe 2001), which respond to mechanical or chemical stimulation, respectively.

Mechanoreceptors

Mechanoreceptors, such as corpuscular endings, Merkel cells, and Meissner's corpuscles, histologically resemble complex neural sheaths (Bradley 2000). They are further distinguished by their location within the laryngeal mucosa as either superficial "touch" receptors or "deep" mechanoreceptors, which appear to be located within the laryngeal muscles or joints (Sampson and Eyzaguirre 1964). Assuming a protective role during normal respiration and swallowing, mechanoreceptors can be spontaneously active, respond to static or dynamic (vibratory) stimuli, and adapt their action potential rate and duration based on the presented stimulus (Boushey et al. 1974; Bradley 2000).

Mechanoreceptors respond to differences in airway pressures (Yu 2005) and can elicit the LCR via increasing airway secretion (Yu et al. 2003), inhibiting inspiration (Yu et al. 2003), and initiating cough (Sant'Ambrogio 1982). Most research available on mechanoreceptors has been performed in the lungs and much is still left to discover about those within the laryngeal mucosa.

Chemoreceptors

Chemosensation is the predominant afferent stimulus responsible for eliciting the LCR. Sensory afferent signals are communicated via chemoreceptors, which are located on the apical surfaces of sensory cells and along epithelial branches of free nerves. As early as 1871, Verson described taste bud-like structures on the laryngeal surface of the epiglottis and the medial and lateral surfaces of the arytenoids (1871). Such laryngeal taste buds were hypothesized to be either a phylogenetic residue or organs of taste "whose chief function is to strengthen the reflexes which close the laryngeal cavity during the passage of food" (Kiesow 1902; Wilson 1905).

Although analogs of the lingual taste bud, laryngeal taste buds likely do not participate in gustation. In fact, stimulating laryngeal taste buds results in eliciting the LCR. For instance, in the fetus and newborn, hypochloremic solutions, acidic solutions, and water can activate laryngeal chemoreceptors and result in startle, rapid swallowing, apnea, laryngeal constriction, hypertension, and bradycardia (Bradley 2000). Ammonia, CO_2, and cigarette smoke are among a list of irritants also known to stimulate chemoreceptors and trigger the LCR (Boushey et al. 1974).

Solitary chemosensory cells (SCC) are elongated slender chemoreceptor cells located throughout the mucosa of the upper airways that express the bitter taste receptor (T2R) along with its associated downstream transduction cascade (i.e., IP_3R3 and α-gustducin). Although there is limited evidence for laryngeal SCCs in humans, those found in the laryngeal mucosa of rodents demonstrate communication with the superior laryngeal nerve (SLN; Smith and Hanamori 1991; Tizzano et al. 2011). Many agonists have been shown to bind to the T2R, including quorum sensing bacterial molecules (e.g., acyl-homoserine lactones) and bitter compounds (Lee et al. 2014). Activation of this cell in the nasal epithelium of mammals results in profound local inflammation via stimulation of its associated sensory nerve, the trigeminal nerve (Lee and Cohen 2014). Based on the taste conduction pathway of the bitter taste cell in the oral cavity, it is thought that activation of the laryngeal T2R within the SCC results in a release of basolateral adenosine triphosphate (ATP; Finger et al. 2005), which then activates local sensory nerves. ATP has been shown to bind to purinoreceptors, which are membrane ion channel receptors consisting of seven subtypes (P2X1–7) and located on sensory nerves (Dunn et al. 2001; Burnstock 2016). P2X2 and P2X3 have been histologically shown to be present in the larynges of mice, rats, and humans. P2X3 immunoreactive nerves have been described as either ramified nerve endings within the epithelium or nerve endings associated with chemosensory cells (Takahashi et al. 2016; Jette et al. 2019).

Free nerve endings, found throughout the laryngeal mucosa from the epiglottis to glottis, including the vocal folds (Widdicombe 2001; Goncalves da Silva Leite et al. 2006; Jette et al. 2019), contribute to chemosensation and can be directly stimulated by various chemicals or via communication with chemosensory cells as described above. Although largely unmyelinated fibers, some of these nerves become

myelinated after they leave the epithelium (Jette et al. 2019). They are immunohistochemically reactive for protein gene product 9.5 (PGP9.5), substance P, calcitonin gene-related peptide, and choline acetyltransferase (Shin et al. 1987; Tanaka et al. 1993; Yamamoto et al. 1997). As with taste buds, the density of these nerve endings also seems to be the highest posteriorly (Wilson 1905; Goncalves da Silva Leite et al. 2016) within the larynx.

A specific type of free nerve ending, the C-fiber, expresses several ion channel receptors that, when activated, elicit airway-protecting mechanisms. C-fibers form a network below the basement membrane of the epithelium and branch extensively and extend into the space between epithelial cells. Stimulation of C-fiber receptors by noxious substances such as capsaicin, bradykinin, citric acid, and sulfur dioxide (SO_2) trigger action potentials in the laryngeal nerves, resulting in cough (Lee et al. 2011; Polverino et al. 2012).

The primary family of ion channels expressed on C-fibers are transient receptor potential (TRP) ion channels. TRPV(vanilloid)1, TRPV3, TRPV4, TRPA(ankyrin)1, and TRPM(melastatin)8 have all been localized to the upper and lower airways and play a vital role in protecting the airway. These receptors activate vagal and/or trigeminal sensory afferents resulting in protective mechanisms such as inflammation, mucous production, airway constriction, coughing, and sneezing (Grace et al. 2014; Bonvini and Belvisi 2017). They are quite promiscuous with regards to their chemical agonists and can be both thermally and mechanically responsive (Table 1). Interestingly, the TRMP8 agonist menthol has been associated with cough suppression (Laude et al. 1994; Preti et al. 2012), though there is inconclusive data to support this claim (Kenia et al. 2008). Alteration in the function and/or expression of TRP receptors has been implicated in chronic airway pathologies such as asthma, COPD, and chronic cough. In these chronic disease states, these receptors partake in a type of feed-forward loop. Initially, they may respond to an exogenous agonist, which can result in local neurogenic inflammation and the release of protrusive mediators (e.g., prostaglandins and bradykinin), which then indirectly bind to and continually act on the ion channel receptors to remain open and active (Grace and Belvisi 2011). There is also evidence of increased expression of these receptors in chronic airway disease which further contributes to associated hypersensitivity (Grace et al. 2014).

Sensory innervation of the larynx

General sensation to the larynx is provided by two predominant branches of the vagus nerve, the SLN and the recurrent laryngeal nerve (RLN). The internal branch of the SLN carries neuronal fibers beneath the thyrohyoid muscle and enters the larynx through the thyrohyoid membrane, providing sensation to the larynx from the epiglottis to the vocal folds. The external branch of the SLN supplies motor fibers to the ipsilateral cricothyroid muscle. The RLN provides sensation to the subglottis and proximal trachea. Afferent impulses are collected and sent via the vagus nerve to the neuronal cell bodies in the inferior vagal ganglion located within the carotid sheath of the neck. These neurons then travel through the jugular foramen in the skull base to enter the brainstem and synapse with neurons in the contralateral spinal trigeminal nucleus. From here, they ascend in the trigeminothalamic tract to the thalamus before arriving at the primary sensory cortex located in the postcentral gyrus.

The internal SLN is the principal sensory nerve of the supraglottis and glottis, though there is some evidence that the RLN and external SLN have minor sensory contributions (Lemere 1932). Communications between the internal SLN and the RLN have been demonstrated in up to 80% of posthumous anatomical studies (Yoshida et al. 1986) either via a communicating branch, such as a Galen's anastomosis (Migueis et al. 1989; Naidu et al. 2012) or a

Table 1. List of TRP family of receptors found in upper and lower airways and their role in cough and hypersensitivity

Receptor	Location expressed	Endogenous agonists	Exogenous agonists	Temperature
TRPA1	Spinal dorsal root ganglia, nasal trigeminal and vagal airway neurons	Bradykinin PGE_2 PAR2 Acid	Allyl isothiocyanate Allicin Cannabinol Acrolein Cinnamaldehyde Curcumin Umbellulone	<17°C
TRPV1	Spinal dorsal root ganglia, nasal trigeminal and vagal airway neurons	Bradykinin PGE_2 PAR2	Resiniferatoxin Capsaicin Vanillitoxins Citric Acid Anandamide	>43°C
TRPV3	Oral and nasal epithelium, skin keratinocytes	2-aminoethoxydiphenyl borate Farnesyl pyrophosphate	Camphor Carvacrol Eugenol Thymol Menthol	31–39°C
TRPV4	Smooth muscle, alveolar wall and septae, pulmonary vasculature, tracheobronchial epithelium, mononuclear cells, alveolar macrophages, and neutrophils	Acid PAR2	Citric Acid	27–35°C
TRPM8	Spinal dorsal root ganglia, trigeminal ganglia, nasal and bronchial epithelial cells		Menthol Eucalyptol Icilin	<15–28°C
TRPC6	Bronchial epithelial cells, alveolar macrophages and pulmonary vasculature	Reactive oxygen species Diacylglycerol	Hyperforin	

PGE2, prostaglandin E2; PAR2, protease activated receptor 2.

plexus of nerves(Migueis et al. 1989), suggesting a potential cross-talk between both the motor and sensory fibers of the recurrent and SLNs.

Stimulation of afferent neurons in the SLN results in action potentials that are relayed to the brainstem. The cell bodies of the afferent neurons are located in the nodose ganglion. Beyond the nodose ganglion, the first synaptic relay occurs in the nucleus tractus solitarius (NTS), a nucleus within the brainstem, which controls respiration, swallowing, and heart rate and rhythm (Reix et al. 2007). Afferent signals are also conveyed to motor neurons of the RLN in the nucleus ambiguous, which activate glottal constrictor muscles, and to phrenic motor neurons in the cervical spinal cord, which inhibit diaphragm contraction and contribute to apnea. Further, the nucleus ambiguous and the NTS incorporate afferent signals from the SLN and send efferent projections to the vagal neurons, which can result in bradycardia, apnea, and glottic closure (Gauda et al. 2007; Thompson 2007).

Ogura and Lam (1953) used subjects with head and neck cancer undergoing laryngectomy procedures who have readily accessible SLN during surgical resection to further investigate the sensory role of the SLN. In each of the four cases studied, the SLN was exposed surgically and stimulated with either a single electrical shock or repetitive shocks of increasing intensities. Patients were asked to describe the sensation as touch, tickle, pain, or taste. Once these intraoperative recordings were completed, a 6 cm section of the nerve from the nodose ganglion to the thyrohyoid membrane was dissected free, stimulated outside the human body for evoked potentials, and further fixed for histopathological analysis. When a single electrical stimulus of 1 ms in duration was applied to the unilateral SLN at low intensities, patients reported a tickle or touch sensation near the larynx but not crossing midline. When this single-stimulus intensity was elevated, patients reported increasing pain. Notably, no swallowing, coughing, or closure of the vocal folds were observed with single stimuli and there were no reports of taste sensation. Repetitive stimuli delivered to the SLN resulted in worse, intolerable pain when compared with the single stimulus. These repetitive stimuli, which were delivered at 30 shocks per second for ¼ s in duration, also resulted in increased mucus production, swallowing, and throat clearing. With increasing intensity of repetitive stimuli, there was abrupt closure of the vocal folds, sharp pain, and even apnea. This study demonstrates that the SLN sensitivity and LCR response is variable depending on frequency and intensity of the stimulus. At higher intensities, there may be recruitment and stimulation of higher threshold fibers, producing stronger motor reflexes, such as throat clearing and apnea.

In studies where the SLN is transected or crushed, the LCR cannot be evoked (Fagenholz et al. 1979). For instance, in anesthetized rats and piglets, denervation of the larynx by section of the SLN, abolished reflexive apneic responses (Fagenholz et al. 1979; Tsai et al. 2009).

The SLN is sensitive to its surroundings and the presenting stimulus and may or may not elicit an LCR accordingly. Physiologically speaking, the larynx is continuously exposed to foreign substances, whether it be through inhalation and swallowing or through refluxate contents with gastroesophageal reflux and vomiting. Regardless of the method of exposure, any stimulation of the larynx can result in activation of LCR, which at times may lead to pathology.

The influence of the LCR: physiologic and pathologic manifestations

Inhalation or ingestion of foreign hazardous substances or regurgitation of gastric contents can result in activation of the LCR. This typically results in cough, an expulsion mechanism to help clear the airways. In the presence of abnormal stimuli, such as local inflammation, gastroesophageal reflux disease (Wilcox et al. 2017), and airborne irritants, the laryngeal mucosa may become hypersensitive. In these scenarios, laryngeal receptors may become abnormally activated resulting in chronic cough, dysphagia, or even life-threatening events such as sudden infant death syndrome (Downing and Lee 1975; Page and Jeffery 2000; Leiter and Böhm 2007; Thach 2008; Praud 2010). Stimulation of the LCR is dependent on the presented stimulus including the method of presentation, concentration and duration, and the local laryngeal environment. The exact physiologic manifestations of the LCR vary according to extrinsic and intrinsic factors, some of which may result in inappropriate activation and persistence of the LCR.

Cough

The laryngeal cough reflex occurs when irritants stimulate C-fiber receptors resulting in action potentials sent to the central "cough center" within the NTS of the medulla. Efferent signals are then sent via the vagus, phrenic, and spinal motor nerves to the diaphragm, abdominal wall, internal intercostals, and laryngeal muscles to elicit immediate expiratory airflow (Polverino et al. 2012). C-fibers are particularly sensitive to physiochemical noxious stimuli such as acids, hyperthermic temperatures, and various airborne chemicals (Polverino et al. 2012).

As described above, the predominant ion channel receptors expressed on C-fibers within the respiratory tract are TRP receptors, of which TRPA1 and TRPV1 predominate (Wallace 2017). The primary role of these receptors is to protect the airway by triggering cough in response to potentially harmful respiratory irritants. TRPV1 receptors are directly activated by aerosolized capsaicin, citric acid, and SO_2 (Lee et al. 2011; Polverino et al. 2012), whereas TRPA1 receptors are directly activated by allyl asothiocyanate (found in mustard oil and wasabi), cinnamaldehyde (cinnamon oil), aldehydes, cigarette smoke, and chlorine (Bandell et al. 2004; Jordt et al. 2004; Andrè et al. 2008; Bessac et al. 2009). Inhalation of capsaicin and citric acid consistently elicit cough in animals and humans in a dose-dependent manner, and consequently, are commonly used in research to objectively measure cough sensitivity (Dicpinigaitis 2003; Dicpinigaitis and Alva 2005; Morice et al. 2007).

Research has shown that patients with idiopathic chronic cough (ICC; a cough persisting for greater than 8 weeks without a known underlying cause) demonstrate hypersensitivity to cough challenges with inhaled capsaicin and citric acid relative to patients without ICC (Millqvist et al. 1998; Doherty et al. 2000; Lee et al. 2011), suggesting increased excitability and/or increased expression of TRPV1 receptors. Groneberg et al. (2004) confirmed increased expression of TRPV1 in patients with ICC versus healthy volunteers using immunostaining of bronchial biopsies. There is also evidence that chronic cough associated with disease states is caused by the local inflammatory response within the airways. For instance, the release of endogenous TRP agonists such as reactive oxygen species and products of lipid peroxidation, and G protein-coupled receptor (GPCR) openers such as prostaglandins and bradykinin, can stimulate vagal neurons and result in cough (Geppetti et al. 2006; Zurborg et al. 2007; Hsu and Lee 2015). GPCRs may also play a modulatory role in airway sensitization. Kwong and Lee (2002) found that pretreatment with prostaglandin E_2 (PGE_2) resulted in increased frequency of action potentials in rat sensory neurons evoked by capsaicin. Similarly, pretreatment inhalation of PGE_2 has been shown to increase sensitivity to capsaicin during cough challenge testing in humans (Stone et al. 1992; Ishiura et al. 2007).

Considering that PGE_2 is produced endogenously during pathologic inflammatory responses, it is likely that cough hypersensitivity seen in patients with chronic inflammatory airway conditions is due in whole or part by a PGE_2-mediated mechanism. This supposition is supported by studies that show an increase in capsaicin-induced cough threshold as a result of PGE_2 inhibitors (Shinagawa et al. 2000; Ishiura et al. 2007).

As mentioned previously, cough is not part of the primary upper airway protective reflex in young infants and newborns, but rather develops gradually over time and is present usually around 1–2 months post term (Miller et al. 1952). Until this time, when evoked, the LCR elicits apnea and glottic closure to protect the airway. When cough is present prior to 1–2 months of age, it suggests an underlying pathology. In human infants with disease, such as an upper respiratory infection or pneumonia, cough has been reported as early as 7 days of age. When compared with healthy newborn infants, premature infants have significantly decreased cough ability (Miller et al. 1952). Yet, as these premature infants age, cough can be elicited more frequently (Fleming et al. 1978). This finding of decreased cough reflex at birth is consistent among other species, including the rat, mouse, rabbit, guinea pig, and dog and is shown to develop over a span of 2–3 weeks (Chang and Widdicombe 2007).

In adults, coughing can be reflexively induced by reducing the chloride ion content of airway surface lining liquid. This can be achieved by inhaling nebulized aerosols of hypochloremic solutions. Pretreatment with inhaled beta-sympathomimetic and anti-muscarinic drugs inhibit this cough reflex, whereas oral beta-sympathomimetics and antimuscarinics have less effect (Higenbottam 1984). Alterations in osmolarity away from iso-osmolarity of inhaled aerosols are a stimulus for bronchoconstriction in subjects with mild asthma. The absence of ions in the presence of iso-osmolarity is not a stimulus for bronchoconstriction, but can be a stimulus for cough (Eschenbacher et al. 1984; Finger et al. 2005; Takahashi et al. 2016; Jette et al. 2019).

The P2X3 receptor, a purinergic receptor found in free nerve endings within the larynx, has been exploited in patients with ICC. A placebo-controlled, double-blind, crossover two-period study using a P2X3 receptor antagonist (AF-219) found that cough frequency was reduced by 75% when patients were allocated P2X3 antagonist compared with placebo. Six patients withdrew from the study secondary to taste disturbances, which were reported by all patients taking the antagonist (Abdulqawi et al. 2015). Applying a P2X receptor agonist to rat larynges also elicited laryngeal hyperreactivity (Tsai et al. 2009). These findings suggest that ATP production in the laryngeal mucosa may provoke the LCR and inhibition along this pathway may allow for various treatment modalities.

Apnea

In infants and newborns, the primary efferent activity of the LCR is glottic closure and apnea. This response is exaggerated and prolonged in premature births across all species (Jadcherla et al. 2006; Reix et al. 2007; Praud 2010). With age, the reflex matures from swallowing and apneic responses to cough. The cause of this exaggerated response in the premature seems to be due to brainstem immaturity as there has been no evidence to suggest alterations in neuronal innervation or laryngeal receptor distribution (Lawson 1981). Yet the exact cause of elicitation remains uncertain as most apneic episodes in infants remain unprovoked.

Apnea is characterized as central, obstructive, or mixed (Matiz and Roman 2003). Central apnea stems from inadequate medullary responsiveness and results in no or poor muscle coordination for breathing. Obstructive apnea is reduced air exchange resulting from obstruction of the airway passages, such as with glottic closure. With obstructive sleep apnea, a vigorous inspiratory effort remains, but is ineffective due to airway obstruction (Eckert et al. 2007; Azagra-Calero et al. 2012). The LCR likely results in a mixed apnea as the reflex causes both the glottis to close and a cessation in effort (Menon et al. 1985).

The LCR can be elicited by hypochloremic or acidic solutions (Thach 2001) such as water or gastric contents. However, physiologically speaking, infants are not normally given pure water and although they have frequent regurgitation, these episodes may not occur prior to apneic events. It has been suggested that prolonged apnea caused by the LCR can result in sudden infant death syndrome(Downing and Lee 1975; Reix et al. 2007; Praud 2010). Downing and Lee (1975) showed that anesthetized piglets and lambs suffered lethal apnea during the instillation of distilled water into the larynx. Even regurgitation episodes have been shown to be associated with mixed and obstructive apnea (Menon et al. 1985). However mucosal stimulation may not be the only contributing factor. As mentioned previously, the LCR can result in systemic changes, such as bradycardia and blood flow redistribution (Thach 2008) and are also prolonged and exaggerated in the premature (Downing and Lee 1975; Reix et al. 2007; Praud 2010). Likely, immature neural processing amongst newborns along with local changes to laryngeal mucosa act in concert to result in prolonged and inappropriate glottic closure with apnea.

Swallowing and dysphagia

The act of swallowing is a complex motor-sensory event that incorporates taste, smell, and sensation of food and liquid to allow for safe consumption. During a swallow, the airway must remain closed such that the bolus entering the esophagus does not enter the trachea. The timing of glottic closure is paramount and any difficulty in sensation, coordination, or strength may lead to aspiration, obstruction of the airway, or choking. Dysphagia is the impairment of normal swallowing and can be present congenitally or acquired through other medical disorders, such as neurological progressive disease, stroke, and cancer (Totosy de Zepetnek et al. 2015).

Taste perception may be important in allowing for efficient and safe swallowing. Logemann et al. (1995) revealed that high citric acid in lemon juice appeared to improve swallowing safety, possibly via stimulation of the lingual sensory nerve, the trigeminal nerve (Logemann et al. 1995; Pelletier and Lawless 2003) or via stimulation of TRPV1 receptors in the larynx associated with the SLN (Lee et al. 2011; Polverino et al. 2012). However, the high concentration of citric acid was perceived poorly among patients as it also resulted in an unpleasant taste and burning. Some also speculate that carbonation or CO_2 may assist in swallowing in those with dysphagia (Jennings et al. 1992; Sdravou et al. 2012), as carbon dioxide is broken down into carbonic acid via carbonic anhydrase, which stimulates the trigeminal nerve. Yet there has been no conclusive evidence to support this claim (Turkington et al. 2017). Although most studies focus on stimulation of the trigeminal nerve with citric acid and carbon dioxide, there is thought that as these substances travel caudally during swallowing, they may also stimulate the SLN and in this way elicit and strengthen airway protection in patients with dysphagia. In fact, we know the SLN contains CO_2 sensitive fibers which may be stimulated via intraepithelial free nerve endings and/or taste receptors (Nishijima et al. 2004). Coates et al. (1996) demonstrated that receptor responses to intralaryngeal CO_2 were abrogated by inhibition of carbonic anhydrase using acetazolamide.

The etiology of dysphagia is multifactorial; however, clinicians can perhaps utilize the protective nature of the LCR to help circumvent aspiration events in these patients.

Inducible laryngeal obstruction

ILO, also known in the literature as paradoxical vocal fold motion, is thought to arise in part to hyperresponsiveness of the LCR in the presence of pathogenic infections, allergy, inflammation, or toxic exposures (Morrison et al. 1999; Hicks et al. 2008). The primary symptom of ILO is variable airway obstruction associated with the inappropriate adduction or closure of the vocal folds. However, patients have also reported associated dry cough, chest tightness, neck or chest retractions, difficulty swallowing, globus sensation (lump sensation), choking, intermittent aphonia or dysphonia, fatigue, and throat clearing (Hicks et al. 2008). Patients with ILO have also been classified with "irritable larynx syndrome" or a "hyperkinetic laryngeal dysfunction" resulting from a triggering stimulus, which often can be benign (Morrison et al. 1999). Many patients with ILO have been observed to have concomitant pulmonary disease, GERD, and sinonasal disease (Smith et al. 2017). Many suspect hyperreactivity or hypersensitivity of the LCR in ILO is secondary to local inflammatory changes resulting in tissue or nerve injury (Morrison et al. 1999), likely from these concomitant disorders. Even inhalational irritant exposure results in local chronic inflammatory changes, as evidenced by lymphocytic inflammation seen in lower airway biopsies of patients with ILO (Altman et al. 2002). Two theories are attempting to explain this inflammatory and injury-driven hyperreactivity. One is the neural plasticity theory. Stemming mostly from the pain literature and studies on C-fiber receptors, this theory suggests nerve or tissue injury provokes afferent inputs to sprout and create new synapses. This growth can allow for a stimulus with a previously known response to elicit an entirely new effect (Morrison et al. 1999). Secondly, repeated local noxious stimulation can alter activation of proto-oncogenes, specifically *fos,* which can result in downstream stimulation or inhibition of neuronal activity and alter a cell's phenotype. If a cell's phenotype is altered, the previously known elicited response from a stimulus may also vary (Morrison et al. 1999).

Laryngopharyngeal reflux: a trigger of the LCR

Laryngopharyngeal reflux (LPR) occurs when acidic gastric contents travel proximally into the laryngopharynx and is commonly implicated in many diseases of the upper and lower airways including otitis media, sinusitis, cough, sleep-disordered breathing, laryngitis, airway stenosis, chronic lung disease, and asthma (Johnston et al. 2016). Aberrant reflexes associated with these diseases may result from chemoreceptor stimulation by refluxate contents. The propensity of upper airway diseases to affect infants and children disproportionately may be explained by the prevalence of reflux during infancy, which peaks at 3–4 months and decreases with increasing chronological age (Page and Jeffery 2000). The role of reflux in dysphagia, chronic lung disease, apparent life-threatening events, apneas, and SIDS is poorly understood (Leape et al. 1977; Herbst et al. 1978).

Pepsin, a digestive enzyme found in gastric refluxate, has been linked to the apneic reflex (Johnston et al. 2016). Pepsin is normally active in the acidic environment of the stomach at a pH of 1.5–2.5. In rats, a study found that apneic reflex was doubled with a pH 5 pepsin, a slightly acidic pepsin. However, pH 7.4 pepsin (neutral), pH 2 pepsin (acidic), and pH 5 denatured pepsin had no effect. Further histological analysis revealed that pH 2 pepsin resulted in the worst histological inflammation albeit without any effect on the reflex arc (Tsai et al. 2006; Johnston et al. 2016). An adult canine model also found that all animals sustained reflexive laryngospasm in response to solutions at a pH of 2.5 or less (Loughlin et al. 1996). Solutions ranging in pH from 4.5 to 8.7, however, did not elicit apnea (Boggs and Bartlett 1982). This research suggests pH of refluxate contents is a possible contributor of LCR activation.

Laryngeal tissues dissected from newborn lambs aged 5–12 days were found positive for Twik-related acid-sensitive K+ channel subtype 1 (Task-1, also known as KCNK3), a pH-sensitive channel responsible for setting membrane potential. The Task-1 protein and mRNA are present in laryngeal mucosa in both ciliated columnar and stratified squamous epithelium (Bournival et al. 2011).

Not only can refluxate alter the sensitivity of the larynx, it can also alter taste. Patients with LPR have been found to have significantly diminished bitter taste scores than those without, though there was no difference for sweet, salty, and sour tastes. Also, there were significantly higher umami scores in the LPR group versus controls, indicating poorer taste function (Altundag et al. 2016). Whether diminished bitter taste in the setting of LPR is due to a change in the expression of bitter taste receptors or dysfunction of bitter taste receptors, or perhaps even overstimulation resulting in adaptation, LPR may alter the SCC's role in the LCR.

Other

In addition to gastric reflux, there are many other physiologic alterations that can result in elicitation of the LCR, either via direct stimulation or alteration in sensitivity of the reflex. For instance, exposure to cigarette smoke (Blair et al. 1996), respiratory virus infection (Weber et al. 2010), anemia, hypoxia, hypercapnia (Xia et al. 2013), and acidosis have all been shown to enhance reflexive apnea (Thach 2008; Praud 2010).

As early as 1983, Haraguchi et al. (1983) noted that the threshold to elicit LCR apnea via SLN stimulation in puppies is reduced in hyperthermic conditions. Neonatal piglets (Curran et al. 2005) and neonatal rats (Xia et al. 2009) have exaggerated laryngeal apneic reflexes in hyperthermic conditions and in intrauterine exposure to cigarette smoke. Both prenatal exposure to nicotine and cigarette smoke exaggerate the hyperthermic prolongation of the LCR (Xia et al. 2009, 2010). Even postnatal exposure to nicotine has been shown to increase apnea in piglets (Frøen et al. 2000) and lambs (Sundell et al. 2003). The enhancement of the LCR by hyperthermia appears to depend on a temperature-sensitive mechanism in or near the nucleus tractus solitarus (NTS) within the brainstem as Xia et al. (2007) showed that focal warming of this region produces an LCR reflex in piglets even when the rest of the animal remains at normal temperature. The pharmacological blockade of $GABA_A$ receptors in the same region prevents the effect of hyperthermia on the LCR (Leiter and Böhm 2007; Xia et al. 2007).

Conclusions

The larynx serves three main functions in humans: maintaining a patent airway, coordinating safe swallow, and contributing to phonation. Its ability to protect the airways is largely due to its reflexive properties. The LCR is present throughout life beginning in the fetus and provides a reflex arc that results in protective physiologic changes. In the fetus and the newborn, elicitation of the LCR primarily produces glottic closure and apnea; yet in the infant and adult, it produces cough. Both glottic closure and apnea and cough

result in airway protection by either preventing passage of foreign substances or expelling them. Although it is mainly beneficial, the LCR can become inappropriately activated or sensitized. It is unclear whether this occurs at the level of the laryngeal mucosa, central integration within the brainstem, or during efferent signaling. However, it is likely a combination of all these components of the reflex arc. There is strong evidence to support that local inflammation either via pathogenic infection or allergy results in a hypersensitivity and persistence of the LCR. Many individuals with infection or allergy also have disorders that are associated with the LCR, such as ICC or ILO. Other disorders, such as dysphagia and aspiration, which can result from whole host of efferent neural disorders, can be treated by stimulating the LCR using carbonation and acid. Much is still to be discovered about the receptors that contribute to the LCR and why certain individuals seem to have a proclivity for inappropriate activation and how we can use the LCR to our advantage in helping to treat multiple disorders affecting the larynx.

Author Roles

Shivani Pathak conceived the concepts in this review and wrote and reviewed the manuscript. Laurie Slovarp conceived the concepts in this review and wrote and reviewed the manuscript. Matthew Clary reviewed the manuscript. Marie Jetté conceived the concepts in this review and wrote and reviewed the manuscript. All authors contributed to the final manuscript.

Conflict of Interest Statement

The authors declare no conflicts of interest associated with this submission.

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