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Update: Pharmacology of Airway Secretion

MATTHEW G. MARIN

Department of Medicine and Environmental Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642

I. Introduction

In 1986, the review, "Pharmacology of Airway Secretion," appeared in this journal (Marin, 1986). My conclusion stressed that "present knowledge only begins to describe the overall pharmacology of airway secretion. Before a complete picture . . . can emerge, we must learn the individual functions and interactions between the multiple cell types of the airways . . . we must understand how pathological mechanisms alter normal airway secretion and how these alterations influence the pharmacology of secretion."

Although considerable progress has been made toward these goals since 1986, we still are far from a complete understanding of the pharmacology of this secretion. The purpose of this update is to delineate major advances in our understanding of this important area that have occurred since the 1986 Review. Some of the research directions that seemed of lesser importance in the mid-1980s have proven to be of greater relevance in the 1990s. I will also review and update these areas.

II. Cellular Structure of Airway Epithelium

The initial review emphasized the cellular complexity of airway epithelium (Marin, 1986). In mammals, 13 cell types had been described in the surface epithelium, nine in submucosal glands, and several others in the submucosal connective tissue. These structural details were reviewed in several reports (Breeze and Wheeldon, 1977; Goco et al., 1963; Reid and Jones, 1979) cited previously. Since 1986, there has been only minimal continued interest in describing the detailed cellular structure of airways; most of this attention has been concentrated on secretory cells. For instance, Robinson et al. (1986) described the secretory cells of the ferret tracheobronchial tree. They noted that the ferret had few goblet cells in the surface epithelium and numerous seromucous submucosal glands. Also of note, the nerve fibers in the surface epithelium of the ferret were sparse. This morphological feature makes the ferret of particular use as a model of airway glandular secretion. Plopper et al. (1986) described the development of submucosal glands in the rhesus monkey. They concluded that submucosal gland development was primarily a prenatal process that continues into the postnatal period and involves a maturation of secretory cells in a proximal to distal sequence. The mucous cells differentiated before the serous cells. Based on the changing proportions of secretory cells during maturation, it is reasonable to predict qualitative changes in airway secretions depending on the age of the subject.

III. Development of Cell Cultures of Airway Cells

To deal with the structural complexity of airways, investigators have recently worked at developing cell cultures of various airway cells. Use of these cultural approaches has permitted important advances in our understanding of the contribution of individual types of airway cells to airway secretion. Potential benefits of cell cultures to the study of airway secretion include an increased amount of study material, greater longevity of

the cultured cells than tissue, ease of handling, and cellular homogeneity (Widdicombe, 1990). All of these advantages have been realized by use of cell cultures derived from airways. In general, there are two types of cell cultures: those derived from submucosal gland cells and those derived from surface airway epithelial cells. The studies in which cell cultures were used are listed in table 1.

A. Submucosal Glandular Cell Cultures

In 1983, Culp et al. (1983) described a technique, involving both enzymatic digestion and mechanical disruption, to isolate submucosal glandular cells from cat trachea. The technique yielded viable, disaggregated submucosal glandular cells. These cells were used for cell cultures (Marin and Culp, 1986; Culp et al., 1992). Cells plated onto glutaraldehyde-fixed gels of rat-tail collagen grew to confluence after 8 days. The cells formed a monolayer of cuboidal cells with ultrastructural characteristics of epithelium and immunoreactivity to antikeratins (suggesting the epithelial origin of the cells). The cells seemed to retain differentiated function in that they synthesized and released radiolabeled high molecular weight glycoconjugates. This secretion was responsive to carbachol stimulation. Of note, the cells were devoid of secretion granules. The cultured cells generated a spontaneous potential difference that could be inhibited by ouabain and increased by carbachol.

Finkbeiner et al. (1986) isolated bovine tracheal glands by enzymatic digestion and were able to propagate serially the cells for 40 passages. The cells grew to confluence and had an epithelioid appearance most compatible with serous cells. They contained numerous osmophilic secretory granules. Maximal growth was achieved when the cells were cultured on human placental collagen-coated culture vessels in fetal bovine serum-supplemented media. The cultures also seemed to retain differentiated functions in that they secreted radiosulfate-labeled macromolecules in response to isoproterenol stimulation. Subsequently, these cell cultures have proven useful for the study of β-adrenoceptors (Madison et al., 1989), the characterization of the synthesis and secretion of an albumin-like protein and lysozyme (Jacquot et al., 1988, 1990), and the characterization of glycoconjugates (Paul et al., 1988). These cell cultures altered their secretory products depending on the composition of the culture media (Jacquot et al., 1990), allowing insights into the modulation of serous cell secretion.

Sommerhoff and Finkbeiner (1990) and Tournier et al. (1990) also developed similar techniques to culture human submucosal gland cells. They found that the cultured cells developed immunocytochemical markers suggestive of both mucous and serous cells, in contrast to freshly isolated cells that possessed markers for one or the other of the two cell types (Sommerhoff and Finkbeiner, 1990). The cultures secreted radiosulfate-labeled macromolecules in response to both cholinergic and adrenergic agonists (Sommerhoff and Finkbeiner, 1990). Using these cultures as a starting point and reculturing on inserts with porous bottoms and coated with human placental collagen, Yamaya et al. (1991a,b) were able to study and contrast the ion transport properties of these cells derived from normal persons and those derived from patients with cystic fibrosis. Tournier et al. (1990) found markers specific to serous cells in their cultured cells, and their cultures were responsive to known secretogogues. Chopra et al. (1991) described the immortalization of human tracheal gland cells by adenovirus simian virus 40. The cells were demonstrated to continue to secrete mucin-like glycoproteins.

TABLE 1
Summary of studies of culturing airway cells

Species and culture	Reference
Submucosal glandular cell	
Cat	Culp et al., 1992
Cow	Finkbeiner et al., 1986
Human	Sommerhoff and Finkbeiner, 1990
	Tournier et al., 1990
	Chopra et al., 1991
Airway surface epithelial cell	
Hamster	Goldman and Baseman, 1980b
	Kim and Brody, 1989
Human	Stoner et al., 1980
	Lechner et al., 1982
	Fiedler et al., 1991
Rabbit	Wu and Smith, 1982
	Liedtke, 1988
Dog	Coleman et al., 1984
Rat	Thomassen, 1989
Guinea pig	Adler et al., 1990a,b
Pig	Tesfaigzi et al., 1990
Ferret	Groelke et al., 1985

B. Airway Surface Epithelial Cell Cultures

Initial attempts to culture airway surface epithelium began in the early 1980s (Goldman and Baseman, 1980a,b). Goldman and Baseman (1980b) used thermolysin treatment and gradient centrifugation to isolate hamster tracheal epithelial cells free of fibroblasts. These cells could be cultured and repeatedly passaged and subcultured. Despite the heterogeneous population of cells used to initiate the culture, the cultured cells had a homogeneous epithelial cell appearance. In a companion paper (Goldman and Baseman, 1980a), these authors showed that their cultured cells secreted a macromolecule with characteristics of a mucous glycoprotein. Because hamster tracheas lack submucosal glands, these authors felt that this secretory function was reflective of goblet cells. Several groups of investigators (Adler et al., 1990a; Kim and Brody, 1989; Kim et al., 1989b) subsequently have described the usefulness of primary cultures of surface epithelium to study mucin secretion by airway surface cells.

In the early 1980s, Stoner et al. (1980) showed that 10

mM putrescine favored the outgrowth of human bronchial epithelial cells relative to fibroblasts in primary cultures. In 1982, Wu and Smith (1982) reported the primary cultures of rabbit tracheal epithelial cells using a defined, hormone-supplemented medium with trace amounts of serum. They confirmed the epithelial nature of their cultures with antikeratin antibodies. Also, in the same year Lechner et al. (1982) published their description of adult human bronchial epithelial cell outgrowth cultures. After 3 to 5 days, the primary outgrowth cultures were dissociated into single cells using trypsin, and these cells were grown in serum-free medium in fibronectin-collagen-coated culture dishes. The cells stained positively for keratin. By 1984, Coleman et al. (1984) developed primary cultures of dog tracheal epithelium which developed electrical properties reminiscent of intact dog trachea. Ultrastructurally, the cultured cells lost their columnar configuration. Welsh (1985), Boucher and Larsen (1988), Widdecombe et al. (1987), and Widdecombe (1990) extended these approaches and demonstrated that these cultured monolayers retained the same cellular mechanisms of ion transport as intact trachea. Liedtke (1988) established primary cultures of rabbit tracheal epithelial cells and showed that these cultures retained differentiated neurohumoral receptor and mediator activity. These cultures increased cAMP in response to β-adrenergic stimulation. Fiedler et al. (1991) used isolated human tracheal epithelial cells and showed that these cells, which were cultured on floating collagen gels, had a sustained production of secretory component.

In 1986, Wu et al. (1986) summarized some of the issues associated with developing differentiated airway epithelial cultures. More recently, numerous investigators have addressed the factors necessary to retain differentiated characteristics in primary cultures of airway cells (McDowell et al., 1987a,b; Van Scott et al., 1988; Thomassen, 1989; Moller et al., 1989; Tesfaigzi et al., 1990; Wu et al., 1990; Gray et al., 1991; Baeza-Squiban et al., 1991; Kondo et al., 1991; Groelke et al., 1985).

C. Conclusions from Cell Culture Studies

Since the early 1980s, numerous approaches have emerged to culture both airway submucosal glandular cells and surface epithelial cells. These experimental models have the potential advantage of permitting study of airway secretory functions unencumbered by the cellular complexity of intact airways. The cultures retain epithelioid morphological features and a variety of differentiated functions, including secretion of mucus-like glycoproteins and secretory component and the transport of ions. The extent to which these cultures truly reflect intact tissue functions still remains to be determined. As will be noted in the following sections, there are other model systems that are also of help in studying airway functions. These include the use of explants of the airway (with and without the surface epithelium intact), freshly isolated airway cells, and whole sections of airways. In summary, these various models have permitted new information to be learned concerning airway secretory processes.

IV. Physiological Regulation of Airway Secretion

To appreciate the pharmacology of airway secretion, it is first necessary to understand the normal functioning of the airways. Recently, some of the new approaches to understanding the cellular mechanisms of airway secretion were reviewed at a National Heart, Lung, and Blood Institute workshop (Basbaum et al., 1988). In the 1986 Review (Marin, 1986) information that was available concerning the nervous regulation of submucosal glandular secretion was described. Since that time, it has been increasingly recognized that nervous innervation of the airways seems likely to play an important role in regulating airway secretion. In recent years there has been considerable enthusiasm for defining the distribution and characterization of nerves present in airway epithelium. In the initial Review, data concerning the ion and water transport functions of surface epithelium were discussed. Further information relevant to the normal secretory physiology of the surface epithelium is included in this section as well.

A. Nervous Regulation of Airway Secretion

Insight into the nervous supply to the airway surface epithelium and submucosal glands provides clues to the manner in which airway secretion is regulated. Four major subgroups of innervation are of relevance: (*a*) parasympathetic cholinergic innervation; (*b*) sympathetic adrenergic innervation; (*c*) nonadrenergic, noncholinergic vagal innervation; and (*d*) afferent innervation of both vagal and spinal origin (Martling, 1987). The recent studies concerning the nervous regulation of airway secretion are summarized in table 2.

TABLE 2
Summary of recent studies of nervous regulation of airway secretion

Species and regulation	Reference
Parasympathetic cholinergic innervation	
Ferret	Baker et al., 1986
Guinea pig	Baluk and Gabella, 1989
Sympathetic adrenergic innervation	
Guinea pig	Baluk and Gabella, 1989
Nonadrenergic, noncholinergic vagal innervation	
Human	Laitinen et al., 1985
Cat	Dey et al., 1988
Ferret	Luts and Sundler, 1989
Guinea pig	Tokuyama et al., 1990
Afferent innervation	
Rat	Spingall et al., 1987 McDonald et al., 1988 Kalubi et al., 1990
Cat	Dey et al., 1990
Guinea pig	Luts et al., 1990

1. Parasympathetic cholinergic and sympathetic adrenergic innervation. A few recent studies have extended knowledge concerning parasympathetic cholinergic innervation. Baker et al. (1986) used whole mounts of ferret trachea, stained histochemically for acetylcholinesterase, and reconstructed the architecture of the nerves and ganglia. Of relevance to airway secretion, these authors observed elaborate superficial nerve plexes over submucosal glands and deep plexes surrounding the acini and ducts of submucosal glands. They identified two types of ganglion cells (I and II). Type II cells were smaller than type I cells, were ellipsoidal, and were located at nodes of the network along the nerves lying over the submucosal glands.

Baluk and Gabella (1989) studied whole-mount preparations of guinea pig trachea. These were stained for acetylcholinesterase, for catecholamines, and for substance P immunoreactivity. Additionally, they used electron microscopy to explore tracheal innervation. The majority of parasympathetic nerves were derived from the vagus via branches of the recurrent laryngeal nerves. Utilizing ligation techniques, they demonstrated that sympathetic fibers from ganglia of the sympathetic chain entered the trachea via small anastomotic branches with the vagus. In some instances, small anastomoses of the sympathetic chain directly supplied the recurrent laryngeal nerve. Distribution of nerves that were immunoreactive for substance P were similar to sympathetic nerves. The authors were unable to distinguish whether both substance P and catecholamines were localized to the same nerves or not.

In summary, these two studies confirmed and extended prior studies that described classical cholinergic and adrenergic nervous innervation of the airways (Richardson, 1979; Basbaum, 1984; El-Bermani and Grant, 1975; Hung et al., 1973; Larsell, 1923; Mann, 1971; Murlas et al., 1980).

2. Nonadrenergic, noncholinergic vagal innervation. Nonadrenergic, noncholinergic innervation of mammalian airways has been recognized since the early 1980s (Barnes, 1984, 1987) and even earlier in amphibian lungs (Campbell, 1971). The neurotransmitter of this innervation has been uncertain but VIP* seems likely (Barnes, 1987). Studies (Dey et al., 1981) from the early 1980s revealed the presence of VIP-immunoreactive nerves in airways of dogs, cats, and humans. Nerve fibers and terminals were observed in several locations, including submucosal glands, smooth muscle, pulmonary and bronchial vessels, and the medial-adventitial junction. This work was extended by Laitinen et al. (1985). They localized VIP-like immunoreactivity in human lower respiratory tract both by light microscopy and ultrastructurally. They also found VIP-like immunoreactivity in nerves supplying bronchial glands. Ultrastructurally, VIP-like immunoreactivity was present in large vesicles (90 to 210 nm).

In 1988, Dey et al. (1988), using immunocytochemical techniques, explored the colocalization of VIP- and substance P-containing nerves in cat bronchi. They found that VIP and substance P colocalized in nerve fibers at several locations, including submucosal glands. At all locations, there were nerve fibers containing only VIP. Only rarely were fibers observed with only substance P. The authors reported two exceptions to the colocalization of VIP and substance P: (*a*) in the airway, surface epithelium fibers contained only substance P; and (*b*) in pulmonary vessels (within the tunica media and close to endothelial layer of the vessel lumen), fibers contained only substance P. Colocalization of VIP and substance P was also noted in nerve cell bodies that made up the intrinsic airway ganglia. The authors stated that this colocalization of VIP and substance P in the same fibers indicated that airway functions, such as glandular secretion, could be partially regulated by the simultaneous or sequential release of VIP and substance P from the same nerve fibers.

Luts and Sundler (1989) studied the nervous supply to the airways of ferrets. They also observed nerve fibers containing VIP supplying the submucosal glands. Nerves containing other peptides, including substance P, calcitonin gene-related peptide, neuropeptide Y, and galanin, were also localized to glandular structures. Also, nerves staining for dopamine-β-hydroxylase (marker for adrenergic nerve fibers) were occasionally observed. The nerves staining for VIP were predominant.

Recently, Tokuyama et al. (1990) studied the neural control of goblet cell secretion in guinea pig airways. Using morphological techniques and electrical vagal stimulation, they showed that goblet cell secretion was under nervous control. They could block this secretion with either atropine or capsaicin pretreatment, leading them to conclude that these goblet cells were under both cholinergic and nonadrenergic, noncholinergic control.

3. Afferent innervation of both vagal and spinal origin. Although prior work, e.g., Davis et al. (1982), suggested that sensory nerves might have a functional effect on submucosal glandular secretion and other airway secretory functions, since the mid-1980s there has been considerable interest in the peptide content of intraepithelial nerve fibers of both vagal and spinal origin. Martling (1987) studied in detail the sensory nerves containing tachykinins and calcitonin gene-related peptide in the lower airways of guinea pigs, rats, cats, and humans. He observed colocalization of substance P, neurokinin A, and neuropeptide K with calcitonin gene-related peptide in nerve fibers lying close to and within the lining airway epithelium of both animals and humans. Substance P and calcitonin gene-related peptide immunoreactivity

* Abbreviations: VIP, vasoactive intestinal peptide; AF-DX 116, 11-[[2-[(diethylamino)methyl]-1-piperidinyl]-5,11-dihydro-6H-pyrido[2,3-b] [1,4]benzodiazepin-6-one; cAMP, cyclic adenosine monophosphate; 5-HETE, 5-hydroxy-6,8,11,14-eicosatretraenoic acid; 15-HETE, 15-hydroxyeicosatretraenoic acid.

were also observed colocalizing in cells of the jugular, nodose, and thoracic spinal ganglia, suggesting a sensory origin. These fibers were shown to belong to the C-fiber group because of their sensitivity to capsaicin.

Springall et al. (1987) investigated the origins of sensory nerves within the rat respiratory tract. They combined retrograde axonal tracing and immunohistochemical techniques and demonstrated that the sensory innervation of the lung originated for the most part in dorsal root ganglia. Almost half of the fibers contained calcitonin gene-related peptide. Most of the calcitonin gene-related peptide immunoreactive nerves in the trachea were derived from the right jugular ganglion.

McDonald et al. (1988) investigated the identity and distribution of nerves that mediated an increase in the vascular permeability of rat trachea, i.e., neurogenic inflammation. They identified that the neurons that mediated neurogenic inflammation had their cell bodies in the jugular (superior sensory) ganglion of the vagus nerve or in the rostral portion of the nodose (inferior sensory) ganglion. They identified a dense innervation of the surface epithelium by sensory nerves that had positive substance P immunohistochemistry. Because of the sparse association of substance P-sensory nerves with the venules affected by neurogenic inflammation, they postulated that the sensory nerves evoked the release of mediators from epithelial cells, which, in turn, contributed to the increased vascular permeability.

Kalubi et al. (1990) provided further evidence that the tracheal epithelium of the rat contained sensory nerve fibers that were immunoreactive for VIP. These nerves were shown, based on ligation experiments and capsaicin pretreatment, to be derived from sensory ganglia of the vagus nerve.

Dey et al. (1990) used immunocytochemical and neuroanatomic methods to show that substance P and calcitonin gene-related peptide were colocalized in the nerve fibers supplying the airway epithelium of cat. These cells were shown to be derived from nerve cell bodies of the nodose ganglion. Luts et al. (1990), studying guinea pig trachea, observed fibers containing both calcitonin gene-related peptide and substance P located mainly within and beneath the surface epithelium and VIP-containing nerves supplying the submucosal glands. Capsaicin treatment indicated that the calcitonin gene-related peptide and substance P nerves were almost exclusively of the sensory type.

4. Conclusions from studies of nervous regulation of airways. Recent investigation has emphasized the complexity of the nervous regulation of airway secretory processes. Submucosal glands are innervated predominately by the nonadrenergic, noncholinergic nervous system, with lesser contributions from the parasympathetic cholinergic and sympathetic adrenergic systems. Although the primary candidate for a neurotransmitter of the nonadrenergic, noncholinergic system is VIP, other peptides also seem likely to have importance. Of particular interest is the role played by sensory nerves belonging to the C-fiber group. These fibers contain a variety of tachykinins and calcitonin gene-related peptide. Stimulation of these fibers elicits submucosal glandular secretion. Based on localization of these fibers, it is suggested that they may also release mediators from airway epithelial cells and, thereby, indirectly have important effects on airway secretory processes, as well.

B. Neurohumoral Receptors on Airway Epithelial Cells

Early studies showing the presence of adrenergic (Barnes and Basbaum, 1983) and cholinergic (Barnes et al., 1982; Basbaum et al., 1984; Marin and Culp, 1986) receptors on ferret tracheal submucosal glands were reviewed previously (Marin, 1986). Several recent studies have investigated both muscarinic and adrenergic receptors on airway epithelial cells and have extended these earlier studies. A few studies have addressed the issue of other types of receptors on these cells. The recent studies concerning neurohumoral receptors on airway cells are summarized in table 3.

1. Adrenergic receptors. Recent studies have extended the knowledge concerning the presence of adrenergic receptors on submucosal glands. Carstairs et al. (1985) used [^{125}I]iodocyanopindolol to label β-adrenoceptors in sections of human lung tissue. They demonstrated that there was specific saturable binding to submucosal glands. Binding was predominantly of the β_2-subtype (90%). Madison et al. (1989) characterized the β-adrenoceptors in cells derived from primary cultures of bovine tracheal gland cells. These cells have characteristics sim-

TABLE 3
Summary of recent studies of neurohumoral receptors on airway cells

Receptor, species, and cell or tissue	Reference
β-Adrenoceptors	
Human/submucosal gland	Carstairs et al., 1985
Human/tracheal surface	Davis et al., 1990
Cow/tracheal submucosal gland	Madison et al., 1989
α-Adrenoceptors	
Cat/tracheal submucosal gland	Culp et al., 1990
Cholinergic receptors	
Cat/tracheal submucosal gland	Culp and Marin, 1986 Ishihara et al., 1992
Cow/trachea	Roffel et al., 1987
Pig/tracheal submucosal gland	Yang et al., 1988
Hen/trachea	Winding and Bindslev, 1990
VIP receptors	
Ferret/tracheal submucosal gland	Lazarus et al., 1986
Gastrin-releasing peptide receptors	
Human/bronchial submucosal gland and surface	Baraniuk et al., 1992
Tachykinin receptors	
Ferret/tracheal submucosal gland	Gentry, 1991
Bradykinin receptors	
Dog/tracheal surface	Denning and Welsh, 1991
Purinergic receptors	
Human/nasal surface	Mason et al., 1991

ilar to serous cells. They also used [^{125}I]iodocyanopindolol to perform radioligand-binding studies of membranes derived from these cells. Binding was saturable, with high affinity, and antagonized by propranolol. Competition with agonists was suggestive of binding to a β_2-adrenoceptor. This group also showed that the secretion of $^{35}SO_4$-labeled macromolecules had a rank order of potency of isoproterenol > epinephrine > norepinephrine. They concluded that the secretory response of these cells was mediated by β_2-adrenoceptors.

Davis et al. (1990), studying surface human tracheal epithelial cells, found that membranes of these cells bound [^{125}I]iodocyanopindolol in a rapid, stereoselective, and saturable manner. The receptor density was calculated at 8.0 ± 4.6 fmol/mg protein (288 receptors/cell). Using selective β-antagonists, the investigators suggested that >90% of the receptors were of the β_2-adrenergic class. In further studies utilizing primary cultures of the cells, the authors examined the cAMP increase as an indication of response to various β-agonists. They observed a rank order of potency of isoproterenol > epinephrine > norepinephrine. The response could be inhibited with a β_2 (either propranolol or ICI 118551), but a not β_1 (atenolol), selective adrenergic antagonist.

Culp et al. (1990) studied the α-adrenoceptors of isolated cat tracheal submucosal gland cells. They demonstrated specific and saturable binding to membranes of these cells by [^{3}H]dihydroergocryptine, an α-adrenergic antagonist with equal affinities for α_1- and α_2-adrenoceptors. They calculated about 19,250 binding sites per gland cell. In competition studies with α_1- and α_2-adrenergic selective antagonists, they demonstrated high- and low-affinity sites for each of the antagonists. This observation was consistent with both receptor subtypes being present on the cells. They also examined the glycoconjugate secretion induced by α-adrenergic agents in "brushed" cat tracheal explants (these explants are free of surface epithelial cells, and secretion is presumed to be from the submucosal glands). They observed the following rank order of potency: norepinephrine ≥ phenylephrine > epinephrine ≫ clonidine. Utilizing various selective α-adrenergic antagonists, they concluded that α_1-adrenoceptors regulate glandular glycoconjugate secretion and that the α_2-adrenoceptors may modulate β-adrenergic stimulated secretion.

2. Cholinergic receptors. Minimal information was presented in the previous Review (Marin, 1986) concerning the role of cholinergic receptors in airway epithelial secretion (Basbaum et al., 1984; Marin and Culp, 1986). Recently, several more articles have appeared (Culp and Marin, 1986; Ishihara et al., 1992; Roffel et al., 1987). Culp and Marin (1986) characterized muscarinic cholinergic receptors in membranes from disaggregated cat tracheal gland cells utilizing [^{3}H]quinuclidinyl benzilate. They observed specific, high-affinity binding to a single population of receptors with about 35,000 receptors per cell. Subsequently, investigators sought to characterize the types of muscarinic receptors present utilizing selective antagonists. The number of subtypes recognized during the ensuing years has increased as further selective antagonists have been applied to the study of airway epithelium (Gross and Barnes, 1988; Minette and Barnes, 1990).

Roffel et al. (1987) characterized muscarinic receptors in a membrane preparation derived from the total bovine trachea, i.e., epithelium and muscle with serosal connective tissue removed. These investigators used radioligand binding with [^{3}H]dexetimide and the muscarine antagonists, pirenzepine and AF-DX 116, to distinguish the subclasses of muscarinic receptors, M_1 to M_3. They presented evidence for 74% of the receptors being of the M_2 subtype and 26% of the M_3 subtype. They speculated that the M_3 receptors were derived from submucosal gland cells.

Yang et al. (1988) characterized the muscarinic receptors of dissociated submucosal gland cells from weanling pigs. They characterized M_1 and M_2 receptors and subdivided the M_2 subtype into M_{2C} and M_{2G} types (equivalent to the M_2 and M_3 subtypes, respectively, described by Roffel et al., 1987). Analysis of competitive binding behavior revealed 27 ± 2% of the M_1 and 73 ± 2% of the M_{2G} subtype of receptor.

Winding and Bindslev (1990) characterized the muscarinic receptor controlling chloride secretion in hen trachea. They used atropine plus five selective antagonists including pirenzepine, hexahydrosiladifenidol, dicyclomine, AF-DX 116, and 4-diphenylacetoxy-N-methylpiperidine methobromide to characterize the hen tracheal receptors. Based on the pattern of antagonism, these authors believed that four subtypes of muscarinic receptors can be identified. They divided the M_3 subtype into two different subtypes: M_3 and M_4. The M_4 subtype, which they demonstrated in hen trachea, is typical of exocrine and mucosal gland cells and has a selectivity profile of 4-diphenylacetoxy-N-methylpiperidine methobromide > atropine ≫ pirenzepine > hexahydrosiladifenidol > AF-DX 116 > dicyclomine. Recently, Ishihara et al. (1992), working with isolated tracheal submucosal glands of cat trachea, examined the effects of pirenzepine, AF-DX 116, 4-diphenylacetoxy-N-methylpiperidine methiodide on methachol-induced, calcium-mediated submucosal gland secretion. They showed that M_3 (probably actually M_4) receptors mediate this type of secretion.

3. Other receptors. Only a few studies have addressed the presence of other types of receptors on airway epithelial cells. This area was reviewed briefly by Barnes (1987).

Lazarus et al. (1986) utilized immunocytological techniques to monitor changes in intracellular cAMP in response to VIP. They observed increases of cAMP in serous and mucous cells of submucosal glands in ferret

trachea and in ciliated and basal cells in dog trachea. They concluded that VIP receptors were present on these cell types.

Baraniuk et al. (1992) performed autoradiographic studies of human bronchi utilizing radioactive gastrin-releasing peptide. They showed binding to both submucosal glandular cells and to surface epithelium. They concluded that there were receptors for this agent in both locations. Gentry (1991) studied the relative potencies of various tachykinins to elicit a high molecular weight glycoconjugate secretory response from explants of ferret trachea. He found a rank order of potency of substance P > physalaemin ≥ eledoisin ≥ neurokinin A > neurokinin B, which is most consistent with the tachykinin receptor type NK1.

Denning and Welsh (1991) studied bradykinin receptors on primary cultures of canine tracheal epithelial cells. Bradykinin increases inositol phosphate, via phosphatidylinositol bisphosphate hydrolysis, and cAMP, via arachidonic acid metabolism. They presented evidence that bradykinin activated the two second-messenger pathways independently and that bradykinin receptors on the apical membrane differed from those on the basolateral membrane, based on differing apparent binding affinities (apical K_D = 39 ± 3 pM, basolateral K_D = 257 ± 53 pM).

Mason et al. (1991) studied the effects of extracellular nucleotides in the regulation of ion transport in primary cultures of human nasal epithelium. Nasal epithelium has similar ion transport properties to airway epithelium (Knowles et al., 1983). Measuring short-circuit current and also intracellular calcium, they determined that the rank order of potency of various purines and pyrimidines was most consistent with the presence of a P_2 purinergic receptor.

4. Conclusions from receptor studies. A variety of neurohumoral receptors are present in airway epithelium. They seem to play a functional role in both submucosal gland secretion, as well as surface epithelial functions. Both α- and β-adrenoceptors have been demonstrated in airway epithelium. On gland cells, β_2- and α_1-receptors regulate glycoconjugate secretion. α_2-Receptors seem to modulate the effects of β-adrenoceptors on gland cell secretion. There is also evidence that β_2-receptors increase cAMP in airway surface epithelium. Specific muscarinic cholinergic receptors are present on submucosal glands which seem to modulate the secretion of glycoconjugates. In addition, there is evidence for the presence of an NK1 receptor on submucosal gland cells, P_2 purinergic receptors on surface epithelial cells, bradykinin receptors on both the apical and basolateral membranes of surface epithelium, and VIP receptors on gland cells, as well as basal and ciliated cells. These and other, as yet undefined, receptors surely play an important role in the pharmacology of airway secretion.

C. Secretory Physiology of the Surface Epithelium of the Airways

The initial Review (Marin, 1986) contained an overview of ion and water transport in airway surface epithelium, and subsequently this area was reviewed by Welsh (1987b). Recently, Duszyk and French (1991) used existing data to provide a new mathematical model of ion movements in airway epithelium. Airway epithelium possesses a sodium-dependent secretion of chloride (Welsh, 1987b). The magnitude of this secretion varies depending on airway generation and species (Welsh, 1987b). Whereas chloride secretion is dominant in dog trachea, sodium reabsorption prevails in the bronchi of dog (Boucher et al., 1981). In rabbit, sodium reabsorption is predominant even in the trachea (Welsh, 1987b). During the last several years, a few studies have contributed additionally to the basic understanding of this area. The recent studies concerning the secretory physiology of the surface epithelium of the airways are summarized table 4.

Cullen and Welsh (1987) studied the regulation of sodium absorption by canine tracheal epithelium. They measured short-circuit current as a reflection of sodium absorption in dog tracheal tissues bathed in chloride-free bathing media. Agents that increased intracellular cAMP (prostaglandin E_2, 2-chloroadenosine, and isoproterenol) increased short-circuit current and presumably sodium absorption. Growth of tracheal epithelial monolayers in aldosterone-containing solution for 2 days resulted in a

TABLE 4
Summary of recent studies of secretory physiology of the surface epithelium of airways

Study, species, and cell or tissue	Reference
Regulation of sodium absorption	
Dog/trachea	Cullen and Welsh, 1987
Cotransport of sodium and chloride	
Cow/trachea	Durand et al., 1986
Rabbit/trachea	Liedtke, 1990
	Liedtke, 1992
Metabolic inhibition of ion transport	
Dog/bronchus	Stutts et al., 1988a
Characterization of ion transport of Clara cells	
Rabbit/bronchus	Van Scott et al., 1989
Characterization of apical chloride conductance	
Human/trachea	Welsh, 1986
	Duszyk et al., 1990
Dog/trachea	Shoemaker et al., 1986
	Li et al., 1990
Cow/trachea	Valdivia et al., 1988
Characterization of apical bicarbonate conductance	
Dog/trachea	Smith and Welsh, 1992
Human/nasal mucosa	Smith and Welsh, 1992
Characterization of active transport of albumin	
Ferret/trachea	Webber and Widdicombe, 1989

50% increase in short-circuit current and a 55% greater inhibition of short-circuit current by amiloride. The authors concluded that sodium absorption can be both acutely and chronically regulated. Furthermore, they speculated "that the stimulation of Na^+ absorption in a Cl^- impermeable [cystic fibrosis] epithelium may have some similarities to stimulation of Na^+ absorption in a normal tracheal epithelium in which Cl^- has been removed. cAMP-mediated stimulation of Na^+ absorption thus might be a normal process in airway epithelia that is uncovered by the Cl^- impermeability in [cystic fibrosis]" (see section V.C.1.a).

Several groups have examined the cotransport of sodium and chloride on the basolateral surface of airway cells. It is hypothesized that active chloride secretion is the result of a coupled Na^+-Cl^- influx across the basolateral membrane, followed by the passive conductance of chloride across the apical membrane. Sodium is thought to be actively pumped from the cell at the basolateral membrane, whereas chloride secretion is a secondary active process. Durand et al. (1986) measured O_2 consumption and short-circuit current simultaneously in bovine tracheal epithelium. Utilizing a variety of maneuvers to alter ion transport, they plotted a stoichiometric ratio of 13.9 ions transported per O_2 molecule utilized, for each sodium, as well as each chloride ion. They suggested that, if there are neither leaks nor recirculation of chloride by other routes, then the observed stoichiometric ratios suggested a 1:1 coupling of Na^+ and Cl^- and not the 1 Na^+:1 K^+:2 Cl^- that has been described in several preparations, i.e., Ehrlich cells, Henle's loop, rectal gland, and colon. Liedtke studied efflux (1990) and uptake (Liedtke, 1992) of radioactive sodium and chloride in epithelial cells isolated from rabbit trachea. Her data was also consistent with 1:1 coupling of Na^+ and Cl^-.

Stutts et al. (1988a) examined the effects of metabolic inhibition (hypoxia or NaCN) on ion transport in the dog. They reasoned that by examining the metabolic dependence of ion conductances they would gain insight concerning the cellular control of the paths for transepithelial ion transport. As expected, they observed that metabolic inhibition decreased net sodium absorption, consistent with inhibition of the basolaterally located Na^+-K^+-ATPase. Metabolic inhibition also increased intercellular permeability as determined by mannitol flux and the backflux of sodium. This suggested that intercellular junctions were under cellular control. Additionally, they demonstrated that metabolic inhibition decreased unidirectional chloride fluxes and the apical membrane chloride conductance. Their data suggested that "the function of 'passive' elements, such as ion channels, can be linked to the function of 'active' elements, such as ion pumps, through a dependence on cellular metabolism."

Van Scott et al. (1989) studied the ion transport of rabbit nonciliated bronchiolar epithelial (Clara) cells grown in culture. They found evidence for sodium absorption from mucosa to submucosa and minimal chloride secretion in the basal state.

Most of the recent interest in the area of ion transport properties of airways has involved further characterization of the apical chloride conductance. As noted in section V.C.1.a, much of this interest has been driven by the discovery of a decrease in this chloride permeability in association with cystic fibrosis. This permeability has been studied primarily through the use of patch-clamp techniques. Welsh (1986) demonstrated in primary cultures of human tracheal epithelial cells an anion-selective channel in apical, cell-free patches that was not strongly gated by voltage or affected by calcium concentration. The channel was blocked by diphenylamine-2-carboxylate and activated by isoproterenol in intact cells.

Canine tracheal epithelial cells maintained in primary culture were studied by patch-clamp techniques by Shoemaker et al. (1986). They identified four anion channel types: (*a*) a highly selective 30- to 50-pS chloride channel, active at negative but not at large positive potentials; (*b*) a highly selective 20-pS chloride channel, active at positive but not at negative potentials; (*c*) a moderately selective, non-voltage-gated 250-pS chloride channel; and (*d*) a moderately selective 10-pS chloride channel, somewhat activated at large negative voltages.

Duszyk et al. (1990) characterized the chloride channels in the apical membranes from primary cultures of human tracheal cells. They found that the predominant channel type had a conductance of 20 pS. The channel had anion selectivity of $NO_3^- > Cl^- > HCO_3^-$. The channel was impermeable to gluconate. Li et al. (1990) studied the anion selectivity of apical membranes of native canine tracheal epithelium using conventional microelectrodes and in primary cultures using patch-clamp techniques. In both instances, they found the following anion selectivity: $SCN^- > I^- > Br^- > NO_3^- \approx Cl^- \gg SO_4^- \approx$ gluconate$^-$. The channel was blocked by 5-nitro-2-(3-phenylpropylamino)benzoate.

Valdivia et al. (1988) purified apical membrane vesicles from tracheal epithelium of cow. They demonstrated the presence of three different chloride channels. The predominant channel, accounting for >80% of the chloride conductance across the vesicles, was characterized as a voltage-dependent, calcium-independent, 71-pS channel. This channel was controlled by phosphorylation in that the mean open time and open probability were increased by in situ exposure to the catalytic subunit of the cAMP-dependent protein kinase.

Recently, Smith and Welsh (1992) utilizing monolayer cultures of canine tracheal and human nasal epithelia provided evidence that there is a sodium-dependent apical conductance of bicarbonate. They suggested that this conductance is via the "chloride channel."

In the past few years there has also been evidence that

albumin is actively transported by the whole trachea of ferret. Webber and Widdicombe (1989) studied the movement of bovine serum albumin across the whole trachea of ferret. They compared albumin movement to the transport of two dextrans (70,000 and 9000 Da). Under baseline conditions the movement of albumin from submucosa to lumen of the trachea exceeded that for the dextrans. They observed that several secretogogues (methacholine, phenylephrine, salbutamol, and histamine) all markedly stimulated the movement of albumin into the lumen relative to the dextrans. Cooling the preparation to 4°C inhibited the albumin transport, which was also shown to be saturable (at an external concentration of 15 $\mu g/\mu l$). All of these factors were suggestive that albumin was actively transported by the trachea and that the rate of transport could be altered by various secretogogues.

1. Conclusions from studies of secretory physiology of the surface epithelium of the airways. In general, airway epithelium possesses a sodium-dependent chloride secretion. The balance between sodium absorption and anion secretion varies among different species and in different airway generations. There is evidence for sodium-dependent bicarbonate secretion and albumin-active transport, as well. This area was reviewed in detail by Welsh (1987b) and recently by Widdicombe et al. (1991). Recently, investigators have examined the coupled entry of sodium and chloride at the basolateral membrane and point to a 1:1 entry ratio. In a number of studies the characteristics of the apical chloride channel have been examined, and four different chloride channels have been identified. The predominant channel type had a conductance of 20 pS and an anion selectivity of $NO_3^- > Cl^- > HCO_3^-$. Evidence indicates that Clara cells actively absorb sodium.

V. Pathophysiology of Airway Secretion

As of the writing of the original Review (Marin, 1986), only limited information addressed the effects of pathological processes on airway secretion. During the past several years new information has emerged concerning the pathophysiology of airway secretion, including the effects of environmental pollutants and other toxins, microbial infection, and various systemic and airway diseases on airway secretion.

A. Effects of Environmental Pollutants on Airway Secretion

A number of articles have been written about the effects of ozone exposure and other pollutants on airway permeability, ion transport, and mucin secretion. The recent studies concerning the effects of environmental pollutants on airway secretion are summarized in table 5.

1. Effects of oxidant stress on airway permeability. Airway permeability is a complex measurement that reflects potentially both transcellular and intercellular conductances of substances across the airways. Alterations in permeability can have important effects on the secretion of water by airway epithelium by regulating the size of the osmotic gradient generated by ion and macromolecular secretory processes (Welsh, 1987b). The studies in which the effects of oxidant stress on airway permeability were examined will be described in this section.

TABLE 5
Summary of recent studies of effects of environmental pollutants on airway secretion

Effects, species, and cell or tissue	Reference
Oxidant stress on permeability	
Rat/nasal mucosa	Bhalla et al., 1986
Rat/trachea	Bhalla et al., 1986
	Bhalla and Crocker, 1986
	Bhalla et al., 1987
	Rasmussen and Bhalla, 1989
	Young and Bhalla, 1992
Rat/bronchus	Bhalla et al., 1986
	Bhalla et al., 1987
	Bhalla et al., 1990
Guinea pig/trachea	Miller et al., 1986
	Stutts and Bromberg, 1987
	Bromberg et al., 1991
Ferret/trachea	McBride et al., 1993
Oxidant stress on ion transport	
Guinea pig/trachea	Stutts and Bromberg, 1987
	Bromberg et al., 1991
Sheep/trachea	Phipps et al., 1986
Ferret/trachea	McBride et al., 1993
Oxidant stress on mucin secretion	
Sheep/trachea	Phipps et al., 1986
Ferret/trachea	McBride et al., 1993
Formaldehyde on ion transport	
Dog/bronchus	Stutts et al., 1986
Human/bronchus	Stutts et al., 1986
Tobacco smoke on mucin secretion	
Cat/trachea	Peatfield et al., 1986

Using radiolabeled diethylenetriaminepentaacetate and bovine serum albumin, Bhalla et al. (1986) studied the relative permeability of nasal, tracheal, and bronchoalveolar mucosa of rats that were exposed to 0.8 ppm ozone in vivo for 2 hours. They measured permeability in vivo immediately and 24 and 48 hours following exposure. They observed a large increase in permeability in the trachea immediately following exposure, which resolved within 24 hours. Immediate injury in the nose was minimal, and it was more profound in the bronchoalveolar areas. The injury persisted for >24 hours in the bronchoalveolar region. Subsequently, they showed that exercise worsened the degree of injury and that the combination of other photooxidant pollutants could also augment the degree of injury (Bhalla et al., 1987). Furthermore, Young and Bhalla (1992) examined the relationship of the influx of polymorphonuclear cells into the airways to changes observed in permeability. They found an increase in polymorphonuclear cells, which lagged temporally behind the peak increase in permeability. Thus, it appeared that the initiation of the in-

creased permeability was not directly related to polymorphonuclear cell influx. Bhalla and Crocker (1986) analyzed the pathway of tracheal permeation following ozone exposure utilizing electron microscopic and autoradiographic approaches. In addition to paracellular conductance, they provided evidence for transcellular vesicular transport of horseradish peroxidase and bovine serum albumin that was accelerated following exposure to ozone.

Using guinea pigs, Miller et al. (1986) examined the effects of a 1-hour exposure to 1 ppm ozone on the permeability of intratracheally instilled horseradish peroxidase. They found increased permeability 2 and 8 hours following exposure. Histamine (0.12 mg/kg, subcutaneously) administered 15 minutes following exposure to ozone augmented the increase in permeability at 2 hours. The authors suggested that this augmentation by histamine resulted from an "enhanced airway response to bronchoactive mediators" due to the oxidant injury.

In a more recent study, Rasmussen and Bhalla (1989) studied the effects of cytochalasin D in vitro on the permeability changes of rat trachea exposed in vivo to ozone. They found that this agent, known to affect the intracellular microfilaments that attach near the cell surface, had a synergistic deleterious effect with ozone exposure on tracheal permeability. They suggested that ozone "may change cell surface structures associated with the microfilamentous cytoskeleton." Bhalla et al. (1990) also performed studies with rat airways and cytochalasin D. They too found results supporting the hypothesis that the cytoskeleton has an important role in maintaining the integrity of airway epithelium following oxidant injury. Bromberg et al. (1991) and Stutts and Bromberg (1987) studied the effects of ozone on airway epithelial permeability using both in vivo and in vitro techniques. They exposed conscious male guinea pigs to 1 ppm of ozone for 3 hours daily for up to 4 days. To make in vivo measurements, they anesthetized the animals and measured the rate of appearance in the blood of radiolabeled mannitol, diethylenetriaminepentaacetate, and inulin instilled into the airways. Ozone exposure markedly increased the rate of appearance of these probes. Furthermore, they showed that instilled horseradish peroxidase was present in the intercellular spaces of tracheal epithelium exposed to ozone but not in control tracheas.

In vitro studies were performed using pieces of trachea that were interposed in Ussing chambers (Ussing and Zerahn, 1951). These excised tracheas demonstrated no increase in permeability after ozone exposure in vivo. The authors postulated that "the effect of ozone inhalation on airway permeability requires the action of mediators that are washed out in the in vitro situation." The investigators also were unable to demonstrate an increase in in vivo permeability after the fourth daily exposure. They were unable to explain the mechanism of this apparent "adaptation" to repeated ozone exposures.

McBride et al. (1993) studied the barrier function of ferret tracheal epithelium by exposing the tissue in vitro to oxidant stress created by the interaction of xanthine oxidase with xanthine in the presence of oxygenated bathing solution. They found that oxidant stress increased the intercellular conductance of ferret trachea as evidenced by an increase in mannitol flux. They also demonstrated that following this stress the paracellular pathway was cation selective.

2. Effects of oxidant stress on ion transport and mucin secretion. In a few studies the effects of oxidants on the ion transport and mucin secretion of airway epithelium have been examined. Bromberg et al. (1991) and Stutts and Bromberg (1987) examined the effects of a 3-hour exposure to 1.0 ppm ozone on ion transport. With guinea pig trachea interposed in an Ussing chamber, they observed that in vivo ozone exposure resulted in an increased short-circuit current due to increased active sodium absorption. Evidence for the cause of increased short-circuit current was circumstantial, based on the ability of apically applied amiloride (a sodium conductance inhibitor) to block the short-circuit response.

Using sheep, Phipps et al. (1986) studied the effects of 0.5 ppm ozone exposure in vivo either acutely (4 hours/day for 2 days), chronically (4 hours/day, 5 days per week for 6 weeks), or chronically with 1 week of recovery. They examined glycoprotein secretion and ion and water fluxes as measured in vitro in an Ussing chamber. They observed that with acute exposure there was an increase in glycoprotein secretion but no change in ion transport. Chronic exposure resulted in an apparent decrease in glycoprotein secretion. However, net chloride secretion and water secretion were augmented. In chronic exposures with 7 days of recovery, glycoprotein secretion was markedly increased, and both the chloride and water secretion were also increased. The authors were unable to demonstrate any significant effect on the electrical conductance across the tissues exposed to ozone.

McBride et al. (1993) working with ferret trachea in vitro observed a transient increase in short-circuit current following enzymatically induced oxidant injury. This response was not inhibitable by furosemide, amiloride, or diphenylamine-2-carboxylate. The authors attributed the response to a transient stimulation of the basolaterally placed sodium pump. Utilizing ferrets, McBride et al. (1991a) also studied the effects of 1.0 ppm ozone in vivo for 24 hours/day for either 3 or 7 days on the measured in vitro secretion of radiolabeled glycoconjugates. They found a 31% increase in basal secretion after 3 days and 11% after 7 days of ozone exposure. Of note, the tracheal gland secretory responsiveness to carbachol was increased. This hyperresponsiveness was attenuated when the animals received treatment with dexamethasone during their exposure to ozone.

3. Effects of other toxicants on airway secretion. In a few recent studies the effect of other toxic agents on

airway secretion was examined. For instance, Stutts et al. (1986) showed that formaldehyde had a deleterious effect on short-circuit current when added to the luminal bathing solution of both dog and human bronchial epithelium interposed in Ussing chambers. They showed that formaldehyde inhibited both sodium absorption and transcellular chloride movement in dog bronchial epithelium. They also showed that formaldehyde resulted in a decreased oxygen consumption. They concluded that their "results are consistent with an action of formaldehyde on cellular ion permeability but effects secondary to metabolic inhibition cannot be dismissed."

Peatfield et al. (1986) studied the effect of tobacco smoke on airway secretion in anesthetized cats. They found that exposure of the trachea or lower airways to cigarette smoke caused an increased tracheal mucin secretion that could be abolished by ganglionic blockade with pentolinium. They demonstrated that exposure to smoke resulted in nicotine being absorbed into the blood. They believed that nicotine stimulated autonomic ganglia that innervate the tracheal submucosal glands and that this was the primary mechanism whereby cigarette smoke augmented tracheal secretion.

4. Conclusions from studies of the effects of environmental pollutants. Pollutants, like ozone, that induce oxidant stress lead to an increase in airway permeability. Although this response is associated with the recruitment of inflammatory cells into the airways, initiation of the response was independent of these cells. Ozone exposure enhanced the reactivity to other agents that also increase airway permeability. The mechanism of the increase in permeability was associated with changes in the microfilamentous cytoskeleton of the cells and also with mediators that "washed out in the in vitro situation" (Bromberg et al., 1991). Following oxidant stress the paracellular pathway favored the movement of cations.

There is also evidence that oxidant stress stimulated both airway ion transport and mucin secretion. The responsiveness of mucin secretion to the secretogogue, carbachol, was augmented following ozone exposure, and this hyperresponsiveness was attenuated by pretreatment with an anti-inflammatory agent, dexamethasone. The mechanism explaining the increased ion transport differed depending on the experimental model used. Agents like formaldehyde and cigarette smoke have varied effects on airway secretion. Formaldehyde decreased ion transport, whereas tobacco smoke augmented mucin secretion.

B. Effect of Microbial Infection on Airway Secretion

Relatively few quantitative studies have appeared concerning the effects of microbial infections on airway secretion. Several studies have addressed the effects of both viral and bacterial infection. These studies are summarized in table 6.

TABLE 6
Summary of recent studies of effects of microbial infection on airway secretion

Effects, species, and cell or tissue	Reference
Viral infection on mucin secretion	
Ferret/trachea	Murray and Jacoby, 1992
Rat/airway	Jany et al., 1991
Viral infection on ion transport	
Cotton rat/trachea	Cloutier et al., 1989
Bacterial infection on mucin secretion	
Guinea pig/airway	Adler et al., 1986

1. Effect of viral infection on airway secretion. a. EFFECT ON MUCIN SECRETION. Gentry et al. (1988) exposed primary cultures of cat tracheal gland cells, prepared by the method of Culp et al. (1992), to influenza A virus. They demonstrated that a productive, nonlytic infection of the cells occurred. The authors speculated, but did not provide direct evidence, that such an infection could alter the secretory properties of the gland cells. Murray and Jacoby (1992) also studied the effect of influenza A viral infection on the secretory response of ferret tracheas. They infected anesthetized adult ferrets by intranasal inoculation with the virus. They measured the secretion of radiosulfate-labeled macromolecules from the tracheas in vitro. Although they demonstrated a virus-induced decrease in the neutral endopeptidase activity, they were unable to show any difference in the baseline macromolecular secretion in control versus virus-infected tracheas. Furthermore, they observed no difference in the responsiveness of virus-infected tracheas to the secretogogue, substance P. They concluded that substance P does not mediate the airway hypersecretory response noted in association with these viral infections.

Jany et al. (1991) studied the expression of a mucin gene in rat airways following both irritation by exposure to 400 ppm sulfur dioxide gas (3 hours/day for 5 days/week for 1 to 3 weeks) and natural infection to Sendai virus. They utilized a human intestinal mucin cDNA, SMUC-41, as a probe. They observed no mucin mRNA in the airways of pathogen-free rats. When the animals were permitted to acquire natural infections, those with antibodies to Sendai (parainfluenza I) virus displayed mucin mRNA in their airways. Animals exposed to sulfur dioxide also expressed mucin mRNA in their airways. The combination of Sendai virus infection and sulfur dioxide exposure resulted in a more intense airway mucin hybridization signal than occurred in rats with only infection. The authors concluded that the mechanism of mucus hypersecretion in infection and airway irritation may be, in part, controlled at the level of mucin mRNA.

b. EFFECT ON ION TRANSPORT FUNCTIONS. Cloutier et al. (1989) examined the effects of respiratory syncytial virus on the bioelectrical properties of tracheas of cotton rats. They exposed the rats to an aerosol of respiratory syncytial virus in vivo and 3 days after exposure studied the bioelectric properties in vitro. By indirect immunofluorescence they showed tracheal infection with respi-

ratory syncytial virus. The infection resulted in a decrease in short-circuit current (attributed to a decreased chloride secretion) and an apparent decrease in paracellular conductance.

2. Effect of bacterial infection on airway secretion. Adler et al. (1986) studied the effect of cell-free filtrates from broth cultures of *Pseudomonas aeruginosa, Hemophilus influenza,* and *Streptococcus pneumoniae* on mucin secretion from explants prepared from guinea pig airways. All three organisms produced substances in broth that stimulated mucin secretion. In the case of *P. aeruginosa* at least three different proteins of 60,000 to 100,000 Da were identified. When isolated by column chromatography, they were heat and trypsin labile and showed proteolytic activity suggestive of proteases. The substances isolated from *H. influenza* and *S. pneumoniae* differed from that isolated from *P. aeruginosa.* These substances were heat labile and 50,000 to 200,000 and 100,000 to 300,000 Da, respectively.

3. Conclusions from studies of the effects of microbial infection on airway secretion. Studies indicated that viral infection can influence both mucin secretion and the ion transport functions of airway epithelium. Influenza A is capable of infecting submucosal gland cells. Although one study failed to demonstrate increased baseline mucin secretion following influenza A infection, the expression of mucin mRNA increased following infection with Sendai virus. This augmentation may regulate, in part, the hypersecretion of mucin associated with that particular viral infection. Infection with respiratory syncytial virus resulted in a decrease in the ion transport of rat trachea and an apparent decrease in the airway paracellular conductance as well. Several bacterial species produced substances, most likely proteases, that stimulated mucin secretion.

C. Effect of Various Diseases on Airway Secretion

Several pulmonary diseases, including cystic fibrosis, asthma, and chronic bronchitis, are characterized by abnormal airway secretory mechanisms. Recent studies of the effects of various diseases on airway secretion are summarized in table 7.

TABLE 7
Summary of recent studies of effects of various diseases on airway secretion

Research area	Reference
Cystic fibrosis	
Regulation of apical chloride conductance	Welsh and Liedtke, 1986 Frizzell et al., 1986 Riordan et al., 1989 Welsh et al., 1989 Boucher et al., 1989 Li et al., 1989 Duszyk et al., 1989 Knowles et al., 1991 Mason et al., 1991
Apical bicarbonate conductance	Smith and Welsh, 1992
Apical sodium conductance	Duszyk et al., 1989
Ion transport of submucosal glands	Yamaya et al., 1991a
Mucin secretion	Rose et al., 1987 Gupta and Jentoft, 1992
Asthma	
Apical chloride conductance in dog model	Duszyk et al., 1991
Bronchitis	
Mucin secretion in rat model	Rogers et al., 1987 Jany et al., 1991 Jany and Basbaum, 1991

1. Cystic fibrosis. a. STUDIES OF ION TRANSPORT PROPERTIES OF CYSTIC FIBROSIS—SURFACE EPITHELIUM. In recent years, the basic knowledge of the pathophysiology of airway secretion in cystic fibrosis has expanded perhaps more than any other area. Recently, the cystic fibrosis gene has been identified on the long arm of chromosome 7 (Riordan et al., 1989; Rommens et al., 1989). The putative gene product is cystic fibrosis transmembrane conductance regulator. Cystic fibrosis transmembrane conductance regulator is likely to be the previously described apical chloride channel (Riordan et al., 1989). In cystic fibrosis, the apical chloride channel has an abnormally decreased conductance (Welsh and Liedtke, 1986). When detached from the airway cell and studied by patch-clamp techniques, the channel itself possessed the same conductive properties as the chloride channel derived from normal cells (Welsh and Liedtke, 1986). The abnormal cystic fibrosis channel also shares a number of regulatory features with chloride channels from normal cells. Welsh et al. (1989) demonstrated that in both types of cells the chloride channels are similarly affected by depolarization, hyperpolarization, change in temperature, and exposure to trypsin. They concluded that "in [cystic fibrosis] cells the conductive properties of the Cl^- channel and all aspects of channel activation appear to be normal except activation by phosphorylation."

There is evidence to suggest a defective regulation of chloride conductance in intact cells from patients with cystic fibrosis. Agents that increase intracellular cAMP, such as isoproterenol, do so in both normal and cystic fibrosis tracheal epithelial cells (Welsh and Liedtke, 1986). However, the increased cAMP in cystic fibrosis cells failed to increase apical chloride conductance as it did in normal cells (Welsh and Liedtke, 1986; Frizzell et al., 1986). This implies abnormal regulation of chloride conductance at a "site distal to cAMP accumulation" (Welsh and Liedtke, 1986).

Alternatively, the calcium-mediated regulation of apical chloride channels was intact in cystic fibrosis cells (Mason et al., 1991; Knowles et al., 1991; Frizzell et al., 1986; Boucher et al., 1989). Boucher et al. (1989) studied the regulation of chloride secretion in human nasal epithelium from normal persons and patients with cystic fibrosis by measuring short-circuit current of amiloride-treated tissues. In normal epithelia, they found that

isoproterenol (β-adrenergic agent, protein kinase A activator), a calcium ionophore (A23187), and phorbol ester (protein kinase C activator) all increased chloride secretion. In cystic fibrosis epithelia only A23187 resulted in an increase in chloride secretion. By examining the additive responses to maximal stimulation with these agents, the authors deduced that isoproterenol and phorbol ester share a common regulatory pathway; whereas A23187 has a separate regulation. Both of the former agents induced an increase in cAMP, but A23187 increased cytosolic calcium. The authors also measured protein kinase activities and observed no difference in the protein kinase A or protein kinase C activity between cystic fibrosis and normal cells. They concluded that their data "suggest a complex mechanism of regulation for Cl^- secretion in human airway epithelia with at least two distinct pathways. . . . agents that activate A and C protein kinases appear to work via a common path that converges on a regulatory protein(s) whose activity is altered by phosphorylation."

There is also evidence for a distinct calcium-dependent path that is uniquely retained in cystic fibrosis tissues. A study by Li et al. (1989) showed that phorbol ester activation of protein kinase C had either a stimulatory or inhibitory effect on chloride secretion depending on calcium concentration. In cell-free membrane patches, protein kinase C inactivated chloride channels at a high calcium concentration (>10 μM) and activated chloride channels at a low calcium concentration (<10 nM). In cystic fibrosis, inactivation, but not activation, by protein kinase C was retained. Thus, the investigators postulated that protein kinase C "phosphorylates and regulates two different sites on the channel or on an associated membrane protein, one of which is defective in cystic fibrosis."

Recent studies by Smith and Welsh (1992) indicated the presence of a sodium-dependent bicarbonate conductance across the apical membrane of airway epithelial cells. Both cytosolic calcium and increased cAMP augmented this conductance in airway monolayers derived from normal subjects, but only calcium caused enhancement in monolayers derived from the airways of patients with cystic fibrosis. The authors suggested that defective bicarbonate secretion via the apical "chloride" channel may also be of importance in the pathogenesis of cystic fibrosis. The retention of a calcium-dependent regulation of a chloride channel in cystic fibrosis provides a potential approach to treatment, i.e., by using extracellular nucleotides that increase cytoplasmic calcium concentration and thereby augment the abnormally decreased chloride conductance associated with cystic fibrosis (Mason et al., 1991; Knowles et al., 1991).

There is also evidence that cystic fibrosis affects more than the regulation of the apical chloride channel. Duszyk et al. (1989) recently examined both chloride and sodium channels in nasal epithelial cells from control subject and patients with cystic fibrosis. They observed a 20-pS nonrectifying channel that was activated by phosphorylation. The channel was more likely to be closed in cystic fibrosis cells than in normal cells. The channel was not activated in cystic fibrosis cells by protein kinase A. Furthermore, the number of 20-pS channels was decreased in the cystic fibrosis cells relative to normal cells, and the numbers of other chloride channels were unchanged or increased in the cystic fibrosis cells. The numbers of sodium channels were increased in the cystic fibrosis cells. The authors concluded that their findings suggested "a wider disturbance of ion channel properties in [cystic fibrosis] than would be produced by a defect in a single channel."

Factors that regulate the abnormal apical chloride channel in cystic fibrosis were recently reviewed in detail by Welsh (1990), and the relevant molecular biology of the chloride secretion and its relationship to cystic fibrosis were reviewed by Rechkemmer (1988). The manner by which the cystic fibrosis transmembrane conductance regulator causes the phenotypic alterations observed in cystic fibrosis remains a matter of speculation. It is suggested that the decreased chloride secretion results in dehydration of the airway secretions resulting in their ineffective clearance (Mason et al., 1991; Knowles et al., 1991).

b. STUDIES OF ION TRANSPORT PROPERTIES OF CYSTIC FIBROSIS—SUBMUCOSAL GLANDS. Yamaya et al. (1991a) studied the ion transport characteristics of tracheal gland cells grown in primary cultures derived from patients with cystic fibrosis and from normal subjects. Confluent cultures were studied in Ussing-type chambers. They observed that baseline transepithelial resistance and short-circuit currents were significantly less in the cystic fibrosis cultures relative to controls. Furthermore, responses to isoproterenol, methacholine, and bradykinin in cystic fibrosis cultures were all attenuated relative to controls. The authors speculated that the responses to the secretogogues reflected an increase in chloride secretion. It was noteworthy that agents that normally act by both increasing cAMP (isoproterenol) and elevating intracellular calcium (bradykinin, methacholine) had their effects attenuated in the cystic fibrosis cultures. The investigators suggested that the chloride channels in the cultured glandular cells may only be activated by cAMP. The increase in chloride secretion in control cultures was speculated to result from an increase in the conductance of the cAMP-dependent channel in the case of isoproterenol stimulation and from an increase of the conductance in the Ca^{2+}-activated basolateral potassium channel in the case of methacholine and bradykinin. Hyperpolarization due to the increased basolateral potassium conductance would increase the driving force for chloride conductance across the cAMP-regulated chloride channel. In cystic fibrosis, in which only cAMP-dependent chloride channels are postulated to be present, chloride

secretion would not increase in response to any of the secretogogues. This speculation remains to be confirmed.

c. STUDIES OF MUCIN SECRETION IN CYSTIC FIBROSIS. The structure of tracheobronchial mucins has been examined in patients with cystic fibrosis compared to that in mucins from control subjects (Rose et al., 1987; Gupta and Jentoft, 1992). Both studies demonstrated that the cystic fibrosis mucin contained more lower molecular weight mucin fragments. However, both investigations, using slightly different approaches, indicated that the observation may have been related to bacterial or inflammatory cell proteases, resulting in a secondary alteration of mucin structure. Both concluded a lack of significant identifiable primary differences between mucins obtained from patients with cystic fibrosis and controls.

2. *Asthma.* It is well recognized that patients dying in status asthmaticus have airways that are blocked by inspissated secretions. Earlier studies by Phipps et al. (1983) demonstrated that sheep allergic to *Ascaris suum* had an increase in mucin secretion and a transient decrease in net chloride secretion in response to *A. suum* antigen exposure in vitro. Despite the importance of this area, minimal additional information concerning the specific effects of asthma or other allergic responses on airway secretion has been forthcoming recently. Duszyk et al. (1991) utilized inside-out patch-clamp techniques on cells derived from primary cultures of dog tracheal epithelial cells. The cells were either from control dogs or dogs sensitized to ragweed. The authors described a new type of chloride channel in the sensitized cells. The channel was voltage gated with maximal opening at about −30 mV. They described the channel as showing inward rectification when bathed in symmetric chloride bathing solutions. The conductance varied from 95 to 52 pS over the range of −60 to +60 mV.

3. *Bronchitis.* The effects of experimentally induced chronic bronchitis on airway secretion have been examined in a few studies. For instance, Rogers et al. (1987) studied the in situ secretion of the sugar, fucose, as a marker for mucous glycoprotein, in the larynx and tracheas of rats exposed to cigarette smoke daily for 2 weeks. These "bronchitic" rats had increased baseline fucose secretion compared with nonexposed control rats. Additionally, acute exposure to cigarette smoke increased fucose secretion transiently. Investigations (Jany et al., 1991; Jany and Basbaum, 1991) have shown that chronic exposure to SO_2 produces a "chronic bronchitis-like" state in rats. Exposure for as little as 1 week in pathogen-free rats induced mucin mRNA hybridization signals that were detected using the SMUC-41 probe. During the week, the rats also showed evidence of Sendai virus infection. Thus, the relative roles of viral infection and irritation secondary to the SO_2 could not be definitely determined.

4. *Conclusions from studies of the effects of various diseases on airway secretion.* Certain disease processes, e.g., cystic fibrosis, asthma, and bronchitis, affect airway secretory processes. Of these, cystic fibrosis is the most fully investigated. Evidence suggested that there is an abnormality of the apical chloride channel associated with its activation by phosphorylation. The site of abnormal regulation is distal to cAMP accumulation. Calcium-mediated regulation of the apical chloride channels remains intact in cystic fibrosis, and this is a feature that is being exploited in developing medical therapies for patients with cystic fibrosis. There is also evidence that the number of apical sodium channels is increased in cystic fibrosis cells. Information is also available concerning the functions of submucosal glandular cells in cystic fibrosis. Although basic mucin secretion was not altered, responsiveness of ion transport to secretogogues was attenuated in cystic fibrosis relative to control cells. It was postulated that only cAMP-dependent chloride channels are present in glandular cells and that these are inactive in cells derived from patients with cystic fibrosis.

Minimal new information is available concerning alterations of secretion present in asthma and bronchitis. There is evidence that sensitized cells may contain a new type of chloride channel. Chronic exposure to irritants such as cigarette smoke or SO_2 can alter the composition of mucin and augment baseline secretion of mucin.

VI. Pharmacological Regulation of Airway Secretion

Investigators have examined the effects of a broad variety of pharmacological agents on submucosal glandular mucin secretion and glandular and surface epithelial cell ion transport under both physiological and pathophysiological conditions. Recent studies of the pharmacological regulation of airway secretion are summarized in table 8.

A. Effect of Cholinergic Agents on Airway Secretion

1. *Effect on submucosal gland secretion.* Studies described in the original Review (Marin, 1986) showed that cholinergic stimulation increased mucin, lysozyme, and electrolyte secretion by submucosal gland cells. As described in sections IV.A.1 and IV.B.2, not only is the nervous supply to submucosal glands well delineated but the distribution of muscarinic receptor subtypes in airways has been investigated extensively. Recent studies of the pharmacology of cholinergic agents have continued to concentrate on submucosal glandular secretion. They have addressed the role played by myoepithelial cells in cholinergically stimulated secretion, the potential down-regulation of cholinergic receptors, the role of anticholinesterase agents, the effect of calcium concentration on cholinergic stimulation, and the effects of cholinergic agonists on glandular ion transport.

a. MUCIN SECRETION FROM SUBMUCOSAL GLANDS. Shimura et al. (1986) developed a technique to mechanically isolate intact single submucosal glands from cat

TABLE 8
Summary of recent studies of pharmacological regulation of airway secretion

Research area	Reference
Cholinergic agents	
Mucin secretion of submucosal glands	Shimura et al., 1986
	Shimura et al., 1987
	Yang et al., 1988
	Gashi et al., 1989
	Ishihara et al., 1990
	Farley and Dwyer, 1991
	Yang et al., 1991
	McBride et al., 1991b
Ion transport of submucosal glands	Yamaya et al., 1991b
Adrenergic agents	
Mucin secretion from submucosal glands	Gashi et al., 1989
	Ishihara et al., 1990
Ion transport of submucosal glands	Yamaya et al., 1991b
Surface epithelial secretion	Liedtke, 1990
	Liedtke, 1992
Active polypeptides	
Mucin secretion of submucosal glands	Borson et al., 1987
	Webber and Widdicombe, 1987b
	Lundgren et al., 1987
	Webber and Widdicombe, 1988
	Webber, 1988
	Lundgren et al., 1990a
Surface epithelial secretion	Rangachari and McWade, 1986
	Tamaoki et al., 1988
cAMP	
Apical chloride conductance	Welsh, 1986
	Widdicombe et al., 1991
Calcium, calcium ionophores, and extracellular nucleotides	
Calcium and submucosal gland secretion	Mian and Kent, 1986
	Kent and Mian, 1987
	Verdugo et al., 1987
	Ishihara et al., 1990
Calcium and ion transport	Welsh, 1987a
	Liedtke, 1990
Extracellular nucleotides and ion transport	Mason et al., 1991
	Knowles et al., 1991
	Galietta et al., 1992
Prostaglandins, their antagonists, and other arachidonic acid metabolites	
Mucin secretion	Johnson et al., 1987
	Kim et al., 1989b
	Hoffstein et al., 1990
	Adler et al., 1990b
	McBride et al., 1992
Ion transport	Widdicombe et al., 1987
	Mochizuki et al., 1992
Anti-inflammatory drugs	
Mucin secretion	Lundgren et al., 1988
	Lundgren et al., 1990a,b
	Shimura et al., 1990
Histamine and antihistamines	
Mucin secretion	Webber and Widdicombe, 1987a
	Webber and Widdicombe, 1989
Diuretics and ion channel inhibitors	
Effect of loop diuretics on ion transport	Liedtke, 1992
Sodium channel inhibitors	Knowles et al., 1990
	App et al., 1990
	Tamaoki et al., 1991
	Acevedo et al., 1991
	Davis et al., 1992
Chloride channel inhibitors	Stutts et al., 1988b
	Schoppa et al., 1989
	Smith and Welsh, 1992
Mucolytic and expectorant agents	
Effects on histological and histochemical appearance of secretory cells	Rogers and Jeffery, 1986
Mucin secretion	Livingstone et al., 1990
Symptomatic treatment of bronchitis	Petty, 1990

and dog tracheas. Utilizing this preparation, with a force transducer, allowed the investigators to examine the contractile response of the myoepithelial cells surrounding the acini of the glands. The workers observed that cholinergic agonists, methacholine and acetylcholine, caused a contraction of myoepithelial cells. This contraction was dependent on calcium concentration in the bathing medium. They suggested that the glandular secretory response to cholinergic agents was partially due to "squeezing of mucus" from the glands by myoepithelial cells. In a subsequent study in which electric field stimulation and autonomic antagonists were used, Shimura et al. (1987) showed that the myoepithelial cells were predominantly under the control of cholinergic nerves. These nerves acted via muscarinic receptors. Phentolamine, an α-adrenergic antagonist, reduced the response to electric field stimulation by only 15%, indicating that α-adrenoceptors have a relatively minor role in contracting myoepithelial cells. There is also evidence that cholinergic stimulation has a direct effect on the mucous cells. Gashi et al. (1989) provided morphometric data from ferret trachea that muscarinic stimulation resulted in mucous cell secretion by the process of exocytosis.

Yang et al. (1988) used both explants of tracheal glands and freshly isolated submucosal gland cells from swine. They examined the effects of acetylcholine on labeled mucin secretion. Acutely, treatment with diisopropylfluorophosphate, a cholinesterase inhibitor, increased the sensitivity to the secretogogue activity of acetylcholine. However, daily pretreatment for 3 or 7 days prior to isolation resulted in a decrease in secretogogue sensitivity to baseline. The authors concluded that "subacute [diisopropylfluorophosphate] treatment leads to a profound loss of responsiveness to muscarinic stimuli." They provided evidence that this was due to "both a decrease

in muscarinic receptor number, particularly of the M_{2G}-receptor type (M_3 receptor) and also a loss of the low-affinity carbechol site."

In a further study with swine tracheal explants, Farley and Dwyer (1991) demonstrated that pirenzepine, an M_3-receptor inhibitor, blocked the mucin secretogogue action of acetylcholine. These investigators (Yang et al., 1991) also showed that subacute treatment of swine with organophosphates (soman, sarin, or VX) for 7 days in vivo inhibited acetylcholinesterase and led to the down-regulation of muscarinic receptors of cells derived from tracheal submucosal glands. They observed that muscarinic density on these cells was reduced by about 65%. This decrease was associated with a decrease in acetylcholine secretogogue activity for mucin.

McBride et al. (1991b) studied the effect of soman, an irreversible anticholinesterase agent, on the secretion of radiolabeled sulfated macromolecules in vitro. Pieces of trachea were mounted in Ussing-type chambers and exposed to various levels of soman on the submucosal side of the tissue. Soman resulted in a concentration-related increase in the secretion of radiolabeled macromolecules with a maximal response occurring at 10^{-7} M. This response could be inhibited with either 10^{-6} M pralidoxime (acetylcholinesterase reactivator) or with 10^{-6} M atropine. At higher soman concentrations, the secretogogue response was not observed. The authors were unable to demonstrate that extremely high levels of acetylcholine attenuated the secretory response. They postulated that the attenuation of the secretory response at high levels of soman related to a "secondary antagonistic response of soman on muscarinic receptors."

Ishihara et al. (1990) isolated glands from cat trachea and demonstrated the effects of methacholine stimulation on the intracellular free calcium concentration. They showed an increase in intracellular free calcium that was dependent, in part, on the availability of extracellular calcium. They suggested that cholinergically induced mucin secretion is mediated by an increase in intracellular free calcium that is derived primarily from extracellular sources.

b. ION TRANSPORT OF SUBMUCOSAL GLANDS. Yamaya et al. (1991b) studied the ion transport properties of primary cultures of human submucosal glands. They observed a chloride secretory response that was markedly responsive to methacholine, a cholinergic agent.

B. Effect of Adrenergic Agents on Airway Secretion

The previous Review (Marin, 1986) emphasized that adrenergic agents have secretory effects on both submucosal glands and the surface epithelial cells of airways. As noted in sections IV.A.1 and IV.B.1, there was evidence for adrenergic nervous supply to submucosal glands, and receptor studies indicated that these cells possess both β- and α-receptors. There is also evidence for β_2-receptors on airway surface epithelial cells (Davis et al., 1990).

1. Effect on submucosal gland secretion. a. MUCIN SECRETION FROM SUBMUCOSAL GLANDS. More recently, Gashi et al. (1989), in a study cited in section VI.A.1.a, examined the effects of adrenergic stimulation on the morphology of tracheal gland mucous cells in ferret trachea in vitro. They observed that isoproterenol (β-adrenergic agonist), but not phenylephrine (α-adrenergic agonist), caused degranulation of mucous cells. These authors concluded that mucous cells, unlike serous cells, are not responsive to α-adrenergic agonists.

Ishihara et al. (1990) measured the intracellular calcium concentration in cat tracheal submucosal glands. They observed that, like cholinergic agents, phenylephrine, but not isoproterenol, increased intracellular calcium. They concluded that α-adrenergic agonists evoke mucin secretion from submucosal glands via an increase in cytoplasmic calcium concentration.

b. ION TRANSPORT OF SUBMUCOSAL GLANDS. As noted in section VI.A.1.b, Yamaya et al. (1991b) studied the ion transport properties of primary cultures of human submucosal glands and observed a chloride secretory response that was weakly stimulated (relative to muscarinic stimulation) by both α- and β-adrenergic agonists.

2. Effect on surface epithelial cell secretion. The effects of adrenergic stimulation on surface airway epithelial cells have been examined recently. Liedtke (1990) investigated the electrically neutral basolateral Na^+-Cl^- cotransport mechanism in rabbit tracheal epithelial cells. She observed that epinephrine activated this cotransport and that this stimulation was inhibited by treatment with the α_2-adrenergic antagonist, yohimbine. Furthermore, an increase in intracellular calcium stimulated this transport. Isoproterenol was without effect. Liedtke concluded that α_2-adrenoceptors stimulate a Na^+-Cl^- basolateral cotransporter and thereby induce a chloride secretory state in these cells. Intracellular calcium was thought to act as a second messenger for this response.

Recently, Liedtke (1992) extended her observations to assess the stoichiometry of sodium and chloride uptake by rabbit tracheal epithelial cells and to determine whether the cotransporter requires potassium. The bumetanide-sensitive uptake of radioactive sodium, chloride, and rubidium into these cells was measured. The investigator showed that the uptake of sodium and chloride, but not rubidium (potassium substitute), was stimulated by α_2-adrenergic stimulation, an increase in intracellular calcium, or a hypertonic bathing media. The stoichiometry of uptake was consistent with 1:1 sodium to chloride coupling and with no role for cotransport of potassium (see section IV.C.).

C. Effect of Active Polypeptides on Airway Secretion

Polypeptides play an important regulatory role over secretory processes in a variety of organ systems. In the

original Review (Marin, 1986) evidence for noncholinergic, nonadrenergic control of submucosal glandular secretion in airways was described. The effects of a variety of polypeptides were summarized. Furthermore, the stimulatory effect of substance P on chloride secretion in dog trachea, studied by Al-Bazzaz et al. (1985), was also delineated. Recent work on the polypeptide content of nerves supplying submucosal glands and surface tracheobronchial epithelium was described in sections IV.A.2, IV.A.3, and IV.B.3, and evidence for receptors for various polypeptides was likewise detailed.

Several articles have appeared that are relevant to the pharmacology of these polypeptides. Investigations have, for the most part, related to the effects of these agents on submucosal glandular secretion and, to a lesser extent, on surface epithelial functions.

1. Effect on submucosal gland secretion. a. MUCIN SECRETION FROM SUBMUCOSAL GLANDS. Borson et al. (1987) extended previous studies of the regulatory role of substance P in submucosal glandular secretion. They explored the manner in which stimulation by substance P could be limited. Using ferret trachea interposed in Ussing chambers, they measured the release of radiosulfate-labeled macromolecules as an indicator of mucin secretion. They demonstrated that substance P induced a dose-related increase in mucin secretion and examined the effects of a variety of inhibitors of proteinases and peptidases on the dose-related secretion induced by substance P. Only the inhibitors of enkephalinase, thiorphan, and phosphoramidon, augmented substance P-induced mucin secretion. The investigators postulated that enkephalinase "degrades [substance P] to inactive metabolites thereby providing an important mechanism for modulating the effects of this neuropeptide on secretion."

Webber and Widdicombe (1987b) examined the effect of VIP on mucin secretion in ferret trachea in vitro. Prior studies by Peatfield et al. (1983) and Coles et al. (1984) were contradictory. Coles et al. (1984) observed that VIP inhibited both baseline and methacholine-induced mucin secretion in explants of human bronchi; whereas Peatfield et al. (1983) observed an increase in mucin secretion in ferret tracheal explants in response to VIP. The apparent contradiction may relate to species differences or, perhaps, to a failure to separate effects on mucous cells from effects on serous cells. Webber and Widdicombe (1987b) measured the volume of tracheal secretion in ferret trachea in vitro. They showed that VIP inhibited the dose-related increase in tracheal secretion induced by methacholine and enhanced the dose-related increase in secretion caused by phenylephrine. These investigators also examined the effect of VIP on lysozyme secretion [a marker for serous cell secretion (Tom-Moy et al., 1983)] induced by methacholine and phenylephrine. Both methacholine and phenylephrine produced dose-dependent increases in the rate of output of lysozyme. In the case of methacholine, the concentration of lysozyme in the total tracheal secretion remained constant; whereas in the case of phenylephrine, the concentration of lysozyme increased in the tracheal secretion. These investigators observed that VIP caused an increased lysozyme concentration in methacholine-induced tracheal secretion, without increasing the rate of lysozyme output. They postulated that this was due to a VIP-induced decrease in the methacholine-stimulated secretion from mucous cells. VIP augmented the rate of lysozyme output due to phenylephrine stimulation without changing the concentration of lysozyme in the tracheal secretion. They suggested that VIP enhances that secretion of serous cells due to phenylephrine.

In a subsequent study, using a similar approach, Webber and Widdicombe (1988) examined the action of a new drug, 4-H-2-carboxamido-4-phenyl-thieno-[3,2c]-[1]-benzopyran. They observed that this agent had an action very similar to VIP, and it had no effect on the basal tracheal mucin secretion from ferret but inhibited methacholine-induced tracheal secretion and augmented phenylephrine-induced secretion. It also had a similar effect to VIP on lysozyme secretion and concentration.

Investigators have also shown that a number of other peptides can induce mucin secretion from either tracheal explant systems or from whole trachea. Lundgren et al. (1990a) studied the effects of gastrin-releasing peptide, a bombesin-like polypeptide, on mucin secretion in a cat tracheal organ culture model. They observed that this peptide caused a dose-related increase in mucin secretion with a threshold response at 10^{-7} M.

Webber (1988) studied the effects of peptide histidine isoleucine and neuropeptide Y on methacholine- and phenylephrine-induced tracheal secretion volume in whole ferret trachea in vitro. Peptide histidine isoleucine inhibited the increased volume of tracheal secretion induced by methacholine but was without effect on the augmentation due to phenylephrine. Peptide histidine isoleucine had no effect on the secretion of lysozyme (serous cell secretion). Neuropeptide Y enhanced both methacholine- and phenylephrine-stimulated tracheal secretion but reduced both methacholine- and phenylephrine-stimulated lysozyme secretion (serous cell secretion). Based on this information, the author suggested that "both [peptide histidine isoleucine and neuropeptide Y] can modulate mucus volume output in the ferret trachea." The overall increase in tracheal secretion volume due to neuropeptide Y was attributed to a stimulatory action "on a mucus source other than serous cells."

Lundgren et al. (1987), using a cat tracheal organ culture system, studied the effects of six opiate peptides (dynorphin, α-endorphin, β-endorphin, γ-endorphin, leucine-enkephalin, and methionine-enkephalin) on mucin secretion. They observed that dynorphin and α-endorphin induced mucin release, and β-endorphin, γ-endorphin, leucine-enkephalin, and methionine-enkeph-

alin were without effect. The response to dynorphin and α-endorphin were both dose related, and maximal responses were noted in concentrations between 10^{-6} and 10^{-5} M. Although naloxone alone was without effect, it blocked the effect of dynorphin in equimolar concentrations. This would imply an action of dynorphin via κ-receptors (Lundgren et al., 1987).

2. Effect on surface epithelial cell secretion. A couple of studies involving polypeptides are of relevance to the secretory properties of the airway surface cells. Tamaoki et al. (1988) examined the effects of neurokinin A and neurokinin B on the bioelectric and ion transport properties of dog tracheal epithelium. Both of these agents induced a dose-related increase in short-circuit current; the response to neurokinin A exceeded that of neurokinin B. The response was more pronounced when the agent was added to a luminal bathing solution. The K_D for luminal addition of neurokinin A was 9 nM, and for neurokinin B, it was 32 nM. The investigators showed that this change in bioelectric properties was related to an increase in chloride secretion toward the tracheal lumen associated with an increase in intracellular cAMP.

Rangachari and McWade (1986) extended previous studies to determine the mechanism of effect of substance P and bradykinin on the ion movement across dog tracheal epithelium. They were particularly interested in the initial decrease in transepithelial potential difference and electrical resistance that occurs immediately after the addition of either substance P or bradykinin to the luminal bathing solution. By manipulating the ionic composition in the luminal and submucosal bathing solutions and clamping the tissues at various potentials, these authors provided evidence that these tachykinins increase the anionic conductance via the paracellular pathway.

D. Effect of Intracellular Cyclic Adenosine Monophosphate on Airway Secretion

Studies reviewed previously (Marin, 1986; Bjork et al., 1982) indicated that a variety of agents, including β-adrenergic agonists, histamine and prostaglandins of the E series, and phosphodiesterase inhibitors, all augment intracellular cAMP. This accumulation resulted in an increased mucin secretion and an increased chloride secretion by airway surface epithelium (Marin, 1986).

Widdicombe et al. (1991) reviewed the central role that cAMP plays in regulating the activity of the apical chloride channel. Welsh (1986) demonstrated that cAMP is involved in the phosphorylation of a channel protein or of a closely associated protein that serves to open the apical chloride channel. Much of the recent information regarding the role of cAMP is described in section V.C.1.a in relation to abnormal regulation of the apical chloride channel in cystic fibrosis and will not be repeated here.

E. Effect of Calcium, Calcium Ionophores, and Extracellular Nucleotides on Airway Secretion

In the prior Review (Marin, 1986), the importance of calcium, both intracellular and extracellular, in influencing the mucin secretion, surface epithelial ion transport properties, and permeability of airways was stressed. More recent studies have extended these initial observations.

1. Effect of calcium on submucosal gland secretion. Calcium plays an important role in regulating the secretion of airway submucosal glands. This observation has been recently confirmed and expanded. For instance, an earlier study by Mian et al. (1982) revealed that increased extracellular calcium concentration can cause mucin secretion by chicken trachea. Subsequently, Mian and Kent (1986) expanded these observations to show that high concentrations of calcium in the submucosal bathing solution (3.6 to 18 mM) or low calcium concentrations in the luminal bathing media (0 to 0.18 mM) can induce secretion of mucins by chicken trachea in vitro. The mucins contained both high ($>2 \times 10^6$) and low molecular weight components (approximately 46,200).

Kent and Mian (1987) also studied transmural calcium fluxes across chicken trachea in vitro. They compared fluxes when the trachea was bathed in physiological calcium concentrations (1.8 mM) versus extracellular calcium concentrations known to elicit mucin secretion, i.e., submucosal bathing solution (3.6 to 18 mM) or luminal bathing (0 to 0.18 mM). Under physiological calcium concentrations, they observed a small secretion of calcium into the tracheal lumen that was augmented by calcium bathing conditions known to augment mucin secretion. The increase was associated with a marked increase in calcium bound to mucin. Thus, mucin secretion seems to be a vehicle for the increased transport of calcium across tracheal cells.

Verdugo et al. (1987) have studied the effects of extracellular calcium on the swelling kinetics of mucin released from goblet cells of rabbit airways. Their data showed that increasing external calcium concentration from 1 to 4 mM decreases the swelling of exocytotically released material markedly and also decreases the diffusion coefficient of the secretory product. They postulated that calcium acts as a "cationic shielding agent" which "screens the negative charges of the mucins, allowing their condensation." When the calcium shield is lost, the negative charges on the mucin induce expansion of the mucin polymer matrix and the observed swelling of the mucin granules. Thus, calcium concentration may be important in determining the viscoelastic characteristics of secreted mucin.

More recently, Ishihara et al. (1990) examined the effects of secretogogues on the intracellular calcium concentration in the acinar cells of cat tracheal submucosal glands. Utilizing the calcium-responsive fluorescent dye fura-2, they studied the dose response and time course

of effect of various secretogogues. They showed that methacholine increased intracellular calcium in a dose-dependent manner with a threshold of 10^{-6} M. The response was inhibited by atropine. Likewise, phenylephrine, at a threshold of 10^{-5} M, induced an increased intracellular calcium concentration that was inhibited by the α blocker prazosin. However, isoproterenol in concentrations of 10^{-7} to 10^{-5} M failed to alter the intracellular calcium.

Neuropeptides had a variable effect on the intracellular calcium, with substance P increasing it and VIP being without effect. The effect of substance P was inhibitable with a cholinergic antagonist, atropine. The authors suggested that in some circumstances calcium may not serve as an intracellular mediator for the secretion of mucin. Several pathways are postulated to be present to mediate the stimulus-secretion coupling in submucosal glandular cells.

2. Effect of calcium on ion transport. Calcium may play an important regulatory role influencing both the coupled Na^+-Cl^- uptake on the basolateral membrane (Liedtke, 1990) and regulation of the apical chloride channel. As described in section V.C.1.a (Boucher et al., 1989), there seems to be a "complex mechanism of regulation for Cl^- secretion" involving at least two distinct pathways: one pathway that converges on a phosphorylation of the chloride channel or associated protein and a second pathway that is activated by increased cytosolic calcium concentration. Welsh (1987a) investigated the events involved in this latter pathway using both native dog tracheal epithelium and primary cultures derived from this epithelium. He showed that mucosal addition of phorbol 12-myristate 13-acetate, an agent that activates protein kinase C, increased chloride secretion in native tracheal epithelium and short-circuit current in both cultured and native tracheal epithelium (reflection of chloride secretion) in a dose-related manner. Half-maximal stimulation was at 10 nM phorbol 12-myristate 13-acetate. Welsh (1987a) also showed that A23187, a calcium ionophore, stimulated short-circuit current via, in part, the production of prostaglandin E_2. This study suggested that activation of protein kinase C and calcium also mediates an increase in the apical chloride secretion. Review of the pertinent studies characterizing these two pathways is described in section V.C.1.a.

3. Effect of extracellular nucleotides on ion transport. Investigators have used extracellular nucleotides (section V.C.1.a) that increase cytoplasmic calcium concentration (Mason et al., 1991) in an attempt to augment the abnormally decreased chloride conductance associated with cystic fibrosis (Mason et al., 1991; Knowles et al., 1991). The role of nucleotides and ion channel regulation was reviewed briefly by Nasmith and Benos (1992) recently.

Knowles et al. (1991) studied activation of chloride secretion by extracellular nucleotides in patients with cystic fibrosis. They measured in vivo changes induced in potential difference across the nasal epithelium of normal subjects and patients with cystic fibrosis during perfusion of the epithelium with amiloride (putatively a reflection of chloride secretion). They applied extracellular ATP and UTP to the apical surface of the cells to potentially bypass the abnormal regulation of chloride secretion. They observed an increase in potential difference in both normal subjects and patients with cystic fibrosis in response to perfusion with ATP. They found that the dose-response characteristics (50% of maximal response at approximately 2 to 4×10^{-6} M) and maximal effective concentration (approximately 10^{-4} M) of both ATP and UTP were similar in both groups. This observation was consistent with a previous study by Mason et al. (1991), indicating that these agents act by means of a P_2 purinergic receptor. It also was suggested that this regulation may relate to an increase in cytosolic calcium, possibly involving "phospholipase C-mediated breakdown of membrane phospholipids to inositol phosphates, coupled with release of Ca^{2+} from internal stores." The maximal effective concentrations of both ATP and UTP were more effective at inducing an increase in potential difference in the patients with cystic fibrosis than in the normal subjects. The investigators, using microelectrodes in cultured nasal epithelial cells, confirmed their in vivo observations that both ATP and UTP could induce an increase in apical chloride conductance in both normal and cystic fibrosis cells.

A recent study by Galietta et al. (1992) expanded these studies. These investigators used the perforated-patch, whole-cell technique to determine membrane currents in 9HTEo-cells, a transformed human tracheal epithelium cell line. They investigated the effects of 2-chloroadenosine and extracellular ATP on Cl^- current and on the mobilization of intracellular calcium. Both 2-chloroadenosine and extracellular ATP activated the movement of chloride. This response could be inhibited by increasing the extracellular osmolality (resulting in cell shrinkage), eliminating extracellular calcium, or adding the A_2 adenosine receptor antagonist, 3,7-dimethyl-1-propargylxanthine. The investigators also studied the role of extracellular and intracellular calcium on these fluxes. In calcium-free bathing media, ATP failed to activate chloride currents, whereas 2-chloroadenosine was able to activate the current.

Galietta et al. (1992) also examined the role of intracellular calcium using furo-2. Both 2-chloroadenosine and extracellular ATP mobilized intracellular calcium, with 2-chloroadenosine achieving a greater mobilization. The authors concluded that the ATP response is much more dependent on extracellular calcium then is the 2-chloroadenosine response. This study differed slightly from that of Mason et al. (1991) in that, in the latter study, UTP had a profound effect on chloride movement, whereas in this study UTP did not activate chloride

current. Likewise, in the Mason et al. (1991) study, there was little response to adenosine, whereas in the study by Galietta et al. (1992) 2-chloroadenosine had an important effect on the chloride current. Thus, the 9HTEo- cells seem to have a different pattern of purinergic receptors from that found in nasal epithelial cells by Mason's group.

Thus, extracellular nucleotides may play an important regulatory role in the apical chloride conductance via the influx of extracellular calcium and by mobilization of intracellular stores. This seems to bypass the abnormality in chloride conductance regulation in cystic fibrosis. However, information is still needed to determine whether this pharmacological manipulation will alter the clinical course of patients with cystic fibrosis. Nevertheless, these observation set the stage for the investigation of extracellular nucleotides as possible therapeutic agents for patients with cystic fibrosis (Mason et al., 1991; Knowles et al., 1991).

F. Effect of Prostaglandins, Their Antagonists, and Other Arachidonic Acid Metabolites on Airway Secretion

In my previous Review (Marin, 1986) a considerable number of studies were examined that related to the effects of prostaglandins, their antagonists, and other arachidonic acid metabolites on airway secretory functions. Recent studies include investigations in which the effects of airway injury and stress on arachidonic acid metabolism and eicosanoid release (Wu et al., 1991; Steiger et al., 1987; Rieves et al., 1992; Doupnik and Leikauf, 1990; Sigal and Nadel, 1988; Leikauf et al., 1988; Freeland et al., 1988; Salari and Chan-Yeung, 1989; Churchill et al., 1989; Hansbrough et al., 1989), the effects of these agents on mucin secretion (Johnson et al., 1987; Adler et al., 1990b; Hoffstein et al., 1990; Kim et al., 1989a; McBride et al., 1992), and the effects on ion secretory mechanisms (Widdicombe et al., 1987; Mochizuki et al., 1992; Eling et al., 1986) were examined. Considerable interest continues to be shown regarding arachidonic acid metabolism, and, recently, pertinent reviews have been published by Sigal (1991) and by Holtzman (1992). The latter review specifically summarized the arachidonic acid metabolites present in a variety of airway epithelial cells and factors that influence eicosanoid release. These studies will be only briefly summarized here. Some of the more important specific citations relative to this area are listed above in this section.

In general, studies reviewed by Holtzman (1992) indicated that different arachidonic acid products are produced, depending on the experimental condition and tissue. For instance, freshly isolated cow and sheep tracheal cells synthesize primarily prostaglandin E_2, whereas canine tracheal cells produce predominantly products of the cyclooxygenase:prostaglandin D isomerase pathway. Human airway cells produce about equal amounts of prostaglandin $F_{2\alpha}$ and E_2. Additionally, Holtzman (1992) pointed out that the maximal and half-maximal activities of the cyclooxygenase pathway occur at lower substrate levels then does the lipoxygenase pathway. He stated that "formation of lipoxygenase products may require substrate concentrations that approach levels toxic to the cell"; whereas prostaglandins may be maximally generated at concentrations of substrate that are lower than that which results in cell injury.

Holtzman (1992) also summarized studies of the lipoxygenase path. He noted that lipoxygenase production in airway cells was also diverse. Human airway cells have uniquely high levels of 15-lipoxygenase pathway products, and the 12-lipoxygenase pathway (canine, porcine, ovine, rabbit) and 5-lipoxygenase pathway (canine, ovine) are more active in other species. For this update, I will concentrate on what is known regarding the specific effects of these agents on airway secretion. The manner in which these products orchestrate the regulation of airway secretion in response to allergic, oxidative, or other cellular injuries remains largely unknown. In the prior Review (Marin, 1986), older studies were summarized that indicate that a variety of prostaglandins (A_2, D_2, E_1, $F_{1\alpha}$, $F_{2\alpha}$, but not E_2) and leukotrienes (C_4 and D_4) cause an increased airway mucin secretion. Recent studies have expanded these prior investigations.

1. Effect on mucin secretion. The effects of arachidonic acid metabolites primarily on submucosal glandular secretion have been examined in several studies. Johnson et al. (1987) used the "hillock" technique to characterize the effects of various inhibitors on the effects of arachidonic acid-induced airway secretion in the dog. The hillock technique (Davis and Nadel, 1980) involves coating the exposed surface of the trachea with finely divided tantalum powder in vivo. Airway secretion is quantified by measuring the rate of appearance of tiny elevations (hillocks) that occur at the gland duct openings in the tantalum layer. The hillocks represent glandular secretion whose accumulation is contained by the tantalum powder. These authors pretreated dogs with indomethacin (cyclooxygenase pathway inhibitor, 5 mg/kg) to attempt to characterize secretory responses due to lipoxygenase products. They injected 1.0 mg arachidonic acid into the cranial thyroid artery to stimulate secretion. Potential inhibitors of secretion were administered either by the thyroid artery or by aerosol. They measured the 15-HETE concentrations in isolated mucus. They showed that antioxidants and inhibitors of lipid peroxidation decreased tracheal airway secretion (induced by arachidonic acid administration). This was correlated with a reduction of 15-HETE production. They suggested a role for lipid peroxidation and 15-HETE production in the regulation of glandular secretion.

McBride et al. (1992) studied the effect of arachidonic acid on the cholinergic secretory responsiveness of ferret tracheal glands. They used brushed tracheal explants in

which the surface epithelium of pieces of trachea was denuded by gentle brushing. They observed that 10^{-3} M arachidonic acid served as a secretogogue for glycoconjugates. Interestingly, the arachidonic acid enhanced the cholinergic secretory response to 10^{-7} M carbachol beyond a simple additive change. This enhancement was partially attenuated by both nordihydroguaiaretic acid (lipoxygenase pathway inhibitor, 10^{-6} M) and indomethacin (cyclooxygenase pathway inhibitor, 10^{-6} M) and was completely inhibited by the combination of the two inhibitors. The effect of arachidonic acid on facilitating the cholinergic stimulation could be abolished by pretreatment of the explants with tetrodotoxin (nerve conduction blocker, 10^{-7} M). The authors concluded that "eicosanoids from the lipoxygenase and cyclooxygenase pathways are involved in this mechanism" and that "this mechanism likely involves the release of an endogenous neurotransmitter."

Other studies are of importance to goblet cell secretion. Adler et al. (1990b) studied the effect of oxygen metabolites on the release of high molecular weight glycoconjugates from organotypic primary cultures of guinea pig tracheal epithelial cells and from explants of guinea pig and rabbit trachea. Secretion from these models would reflect primarily surface goblet cells because well-developed submucosal glands are not present in these species. Oxygen metabolites were generated by the catalysis of purine by xanthine oxidase. They showed that there was a dose-related increase in glycoconjugate secretion in response to oxygen metabolites. Further analysis of the bathing solution of cell cultures following exposure to oxidant stress revealed an increase in prostaglandin $F_{2\alpha}$. Inhibition of this increase in prostaglandin $F_{2\alpha}$ by metabolite scavengers or by inhibitors of arachidonic acid metabolism also inhibited glycoconjugate secretion. Other arachidonic acid metabolites, such as 5-HETE and 15-HETE, were also increased following oxidant stress. However, in contrast to the findings of Johnson et al. (1987), only prostaglandin $F_{2\alpha}$ induced an increased in glycoconjugate secretion in the models of Adler et al. (1990b). Adler and coworkers suggested that oxygen metabolites increased airway glycoconjugate secretion via the production of prostaglandin $F_{2\alpha}$.

Hoffstein et al. (1990) used a morphometric approach to examine the effect of leukotriene D_4 on goblet cell secretion from guinea pig trachea. The guinea pigs were exposed in vivo to various concentrations of leukotriene D_4 delivered by aerosol, and the fractional volume of mucous granules (staining with Alcian blue-periodic acid Schiff) was quantitated in sections taken from the airways. A dose-related increase in the fractional volume of mucous granules in tracheas exposed to aerosol concentrations was found between 0.1 and 1 μg/ml, with a maximal response achieved at a concentration of 1 μg/ml. The authors suggested that leukotriene D_4 is important in goblet cell secretion.

Kim et al. (1989b) also examined the mechanisms involved in mucin secretion by airway goblet cells. They used a primary culture of hamster tracheal epithelial cells. At confluence these cultures appeared to be primarily secretory cells and produced mucins like goblet cells. They were unable to augment mucin secretion with either prostaglandin E_2 or $F_{2\alpha}$ (10^{-9} to 10^{-5} M). Likewise, leukotrienes C_4 and D_4 in "physiological" concentrations of 3×10^{-8} to 3×10^{-6} M failed to increase secretion but did so at a "pharmacological" concentration of 3×10^{-5} M. Interestingly, factors that induced secretion were hypo-osmolarity (200 mosM), decreased (4.0) and increased (9.0) pH, and the neutrophil proteases, elastase and cathepsin G.

Thus, submucosal glandular mucin secretion seems to be influenced by arachidonic acid metabolites from both the lipoxygenase and cyclooxygenase pathways, and some evidence indicates that these products may act via the release of neurotransmitters (Johnson et al., 1987; McBride et al., 1992). The conclusions are less clear for the effects of arachidonic acid metabolites on goblet cell secretion. Whereas lipoxygenase and cyclooxygenase pathway metabolites may have an effect on goblet cell secretion (Adler et al., 1990b; Hoffstein et al., 1990), the experiments of Kim et al. (1989b) raise questions regarding the pathophysiological significance of these responses. The importance of these questions depends in large measure on how relevant the various culture models are to the in vivo condition. At this point, further studies seem necessary to define the true pathophysiological role of arachidonic acid metabolites on goblet cell mucin secretion.

2. Effect on ion transport. The prior Review (Marin, 1986) summarized studies that showed that both prostaglandins $F_{2\alpha}$ and E_1 increased dog tracheal chloride secretion; prostaglandin E_1 decreased sodium absorption as well. Pretreatment of tissues with indomethacin reduced chloride secretion to a more reproducible and stable baseline, presumably by blocking endogenous prostaglandin synthesis. Addition of exogenous prostaglandins still elicited a response after indomethacin pretreatment. A few recent studies have built on these prior observations and are of relevance to the regulation of ion transport by the airways.

Widdicombe et al. (1987) developed primary cultures of dog tracheal epithelium and showed that these cultures increased short-circuit current in response to prostaglandin E_2 and $F_{2\alpha}$ and to leukotrienes C_4 and D_4. Eling et al. (1986) isolated dog tracheal epithelial cells and measured arachidonic acid metabolism in the cells. The cells were stimulated with the calcium ionophore, A23187, and they measured metabolites in the incubation media. The primary metabolite was prostaglandin D_2 (14.5 ± 4.5% of total metabolites), leukotriene C_4 (10.2 ± 2.5%), and leukotriene B_4 (12.8 ± 2.5%). The investigators also measured the short-circuit current response of canine

tracheas pretreated with indomethacin and measured the relative potencies of arachidonic acid metabolites on increasing presumptive chloride secretion. They observed that all of the prostaglandins tested (H_2, D_2, E_2, $F_{2\alpha}$, I_2) increased short-circuit current. The most effective were prostaglandins H_2, D_2, and E_2. They also tested lipoxygenase products, leukotriene C_4, B_4, D_4, and 5-HETE. Of these, leukotriene C_4 was the most effective in increasing short-circuit current, with a small response observed even at 10^{-11} M. Leukotriene B_4 showed only a minimal effect, and leukotriene D_4 and 5-HETE were without effect. The authors also showed that the short-circuit response to A23187 corresponded to the time course of prostaglandin D_2 release. They suggested that the airway epithelial cells, by producing these arachidonic acid metabolites, "may play an important role in modulating functional activities that control the volume and composition of airway secretions." They believed that the endogenous production of prostaglandin D_2 was an important factor in the regulation of ion transport secretory functions of airways.

Mochizuki et al. (1992) studied the effects of arachidonic acid (5×10^{-5} M) on chloride secretion in primary cultures of dog tracheal epithelium. To prevent the metabolism of the arachidonic acid to metabolites known to stimulate chloride secretion, they utilized 5×10^{-6} M of both indomethacin and nordihydroguaiaretic acid, inhibitors of the cyclooxygenase and lipoxygenase metabolic pathways, respectively. In the absence of the inhibitors, arachidonic acid increased short-circuit current as expected by 27.8 ± 5.2 $\mu A/cm^2$; in the presence of the inhibitors, addition of arachidonic acid decreased short-circuit current by 4.4 ± 0.6 $\mu A/cm^2$. This effect was not changed by the addition of amiloride, a sodium channel blocker. In the presence of arachidonic acid plus the two inhibitors, indomethacin and nordihydroguaiaretic acid, the short-circuit current responsiveness to isoproterenol was attenuated. Studies were also performed in which the efflux of ^{125}I in high potassium media from these cells was examined. This halide leaves the cells only via the apical chloride channels. The investigators showed that arachidonic acid plus the two inhibitors decreased baseline efflux of ^{125}I by 50%. This treatment also reduced the increase in efflux induced by isoproterenol to 20%. The authors interpreted their data to indicate that arachidonic acid itself has an inhibitory effect on apical chloride channels.

G. Effect of Anti-Inflammatory Drugs on Airway Secretion

Despite the clinical impression that corticosteroids reduce bronchorrhea, there have been relatively few quantitative studies of the effects of steroids or other anti-inflammatory agents on airway secretory mechanisms. Jeffery (1986) reviewed early studies that showed that a variety of anti-inflammatory agents can prevent the development of morphological changes suggestive of bronchitis. In these studies, either indomethacin, flurbiprofen, dexamethasone, prednisolone, hydrocortisone, or a mucolytic drug, N-acetylcysteine, were given daily for 2 weeks to pathogen-free rats exposed to cigarette smoke. These treatments prevented morphological changes suggestive of bronchitis that occurred in untreated control animals. Recently, Lundgren et al. (1990b) reviewed the various mechanisms by which glucocorticoids inhibit the secretion of mucus in an inflammatory state, e.g., asthma. They enumerated the multiple points at which steroids prevent the release of inflammatory mediators, many of which have been demonstrated to have secretogogue effects. During the last 10 years, several reports have appeared that relate directly to the effects of anti-inflammatory agents on mucin secretion.

In 1984, Marom et al. (1984) examined the dose-related effects of dexamethasone and methylprednisolone on the release of radiolabeled glycoconjugates from human airway explants cultured for 34 hours. Both of the steroids caused a dose-related inhibition of glycoconjugate release, with inhibition first noted after 8 hours of incubation and a maximum reached by 16 to 24 hours of incubation. By 34 hours, inhibition was no longer observed. In the case of dexamethasone, the threshold for inhibition was at 10^{-9} M; methylprednisolone had a threshold of 10^{-7} M. These investigators also examined the effects of dexamethasone pretreatment on secretogogue-induced glycoconjugate secretion by histamine and 5-HETE. The steroid pretreatment reduced the secretory response in both cases by lowering baseline secretion.

Lundgren et al. (1988) studied the effect of dexamethasone on glycoconjugate secretion from cat tracheal explants. Dexamethasone caused a dose-related reduction in glycoconjugate release after 16 hours of incubation, with maximal reductions noted at 24 to 64 hours. If dexamethasone was removed from the incubation media, the rate of secretion gradually returned to control levels. The time for return depended on the concentration of dexamethasone used. The investigators also measured lipocortin levels in the tracheal tissue. Lipocortin is an inhibitor of phospholipase. Induction of this protein prevents the cleavage of arachidonic acid from cell membranes and the initiation of the cyclooxygenase and lipoxygenase metabolic pathways. Treatment of the tracheal explants with dexamethasone resulted in a progressive increase in lipocortin concentrations during a 24-hour period. This suggested the possibility that the mechanism of inhibition was related to lipocortin induction. In support of this contention the investigators observed that addition of a murine monoclonal antibody to lipocortin prevented, in a dose-related manner, the inhibitory effect of dexamethasone. Furthermore, addition of exogenous arachidonic acid (100 μg/ml) in dexamethasone-treated explants reversed the dexametha-

sone-suppressed secretion, providing additional support for the role of lipocortin. The authors speculated "that a portion of the therapeutic effect of glucocorticoids in respiratory mucus hypersecretion is related to the induction of the synthesis of lipocortin, which can inhibit arachidonic acid release and eicosanoid formation."

Shimura et al. (1990) examined the effect of dexamethasone on precursor uptake, biosynthesis, and release of radiolabeled glycoconjugate from cat tracheal glands. These investigators isolated individual glands from cat trachea and incubated them with D-[6-^{3}H]glucosamine, a precursor of mucous glycoprotein, for 16 hours. They measured the release of incorporated label. Treatment with dexamethasone (10^{-9} to 10^{-5} M) for 24 to 72 hours resulted in a dose-dependent decrease in radiolabel release with a maximum of a 78% decrease. They also examined the amount of radiolabel in dissolved gland tissue and observed that this was increased by dexamethasone treatment. They also showed that dexamethasone treatment (10^{-5} M for 48 hours) decreased, relative to untreated glands, the release of radiolabeled glycoconjugates in response to dibutyryl cAMP (50 mM), phenylephrine (10^{-5} M), and fenoterol (10^{-5} M). The ratio of secreted to intracellular radiolabel also decreased with dexamethasone pretreatment. The investigators concluded that glucocorticoids inhibited both baseline and stimulated glycoconjugate secretion by tracheal submucosal glands and that the inhibition was due to a decrease in the release from the glandular cells and not due to alterations in either precursor uptake or biosynthesis.

H. Effect of Histamine and Antagonists on Airway Secretion

The effects of histamine on airway secretion have been examined in only a few prior studies. As reviewed previously (Marin, 1986), the effects on mucin secretion were equivocal (Chakrin et al., 1973; Richardson et al., 1978) and the effect on ion transport was to increase chloride secretion and to decrease sodium reabsorption (Marin et al., 1977; Vulliemin et al., 1983). This effect was competitively inhibited by diphenhydramine (H_1 antagonist) but not by burimamide (H_2 antagonist).

More recently, Webber and Widdicombe (1987a) studied the effects of histamine on liquid secretion from ferret trachea in vitro. They collected secretions carried by gravity from the end of the trachea and determined the volume of secretion and the lysozyme concentration (a reflection of serous cell secretion). They found that histamine (0.3 mM) increased the volume of airway secretion and lysozyme output (but not concentration) compared with controls. The authors concluded that histamine is a potent stimulator of serous cell secretion. Because the lysozyme concentration remained constant, the authors suggested that histamine also increased output from mucous cells as well. Using the same system, this group (Webber and Widdicombe, 1989) also studied the effects of histamine on albumin transport across ferret trachea. They showed that histamine increased not only mucus secretion but also albumin output and concentration.

I. Effect of Diuretics and Ion Channel Inhibitors on Airway Secretion

The usefulness of diuretic agents and ion channel inhibitors to study the ion transport properties of airway tissues was emphasized in the prior Review (Marin, 1986).

1. Effect of "loop" diuretics. The effects of furosemide and other "loop" diuretics on the ion transport properties of dog trachea were examined in a number of previous studies (Marin and Zaremba, 1979; Welsh, 1983; Widdicombe et al., 1983), and an approximately equal decrease in the influx of sodium and chloride into the tracheal cells was noted. More recently, Liedtke (1992) used bumetanide, a loop diuretic, to assess the stoichiometry of sodium and chloride uptake by rabbit tracheal epithelial cells and to determine whether the cotransporter requires potassium. These studies were described in section VI.B.2.

2. Effect of sodium channel inhibitors. The use of amiloride, an inhibitor of sodium conductance, has been used to demonstrate the presence of a sodium conductance across the apical cell membrane of tracheal cells (Welsh et al., 1983; Estep et al., 1982). Furthermore, the effects of agents that seem to act via an effect on the apical sodium conductance have been examined in several studies. For instance, Tamaoki et al. (1991) studied the effects of ambroxol, *trans*-4-[(2-amino-3,5-dibromobenzyl)amino]cyclohexanol hydrochloride, on the ion transport functions of cultured canine tracheal epithelial cells. This is a drug reported to have several pulmonary effects, including increasing surfactant secretion and decreasing inflammation. The investigators found that ambroxol caused a dose-related decrease in short-circuit current when added to the submucosal, but not the luminal side, of the cultures. The half-maximal response was obtained at a concentration of 60 nM. Because this response could be inhibited by pretreatment with amiloride, but not with bumetanide or diphenylamine-2-carboxylate (chloride channel inhibitor), the response of ambroxol was attributed to a decrease in sodium conductance in the luminal membrane. Because ambroxol did not have an effect when added to the luminal membrane, the workers believed that the response was not a direct effect on the sodium channel. They speculated that it was mediated via "intracellular signal transduction systems."

Acevedo et al. (1991) examined the effects of amiloride and benzamil, a higher affinity sodium channel blocker. They utilized sheep trachea which has a predominant net sodium absorption. Using microelectrodes, they determined the apical membrane potential, voltage divider ratio, transepithelial potential, and transepithelial re-

sistance. A 20-second exposure to either amiloride (10^{-5} M) or benzamil (3.8×10^{-7} M) hyperpolarized the apical membrane and increased the voltage divider ratio. A 1-hour exposure to benzamil resulted in no significant change in either apical membrane voltage or the voltage divider ratio. Circuit analysis showed that the tissues treated with benzamil had increased apical and basolateral membrane resistances. The authors suggested that there is "a decrease in basolateral potassium conductance secondary to the initial decrease in apical sodium conductance." This, they postulated, caused the restoration of the electrical driving force for other ions across the apical membrane in the presence of hyperpolarization of the apical membrane.

In recent studies amiloride was used to alter the abnormal ion transport observed in cystic fibrosis and thereby possibly ameliorate the lung disease (Knowles et al., 1990; App et al., 1990). Knowles et al. (1990) performed a double-blind, crossover study in which the effects of aerosolized amiloride for 25 weeks on (among other variables) the sputum viscosity and elasticity of patients with cystic fibrosis were examined. Utilizing a magnetic oscillating sphere, they observed a significant decrease in indexes of the mechanical impedance of mucus. They also described an augmentation of mucociliary clearance and cough clearance of mucus in patients during treatment with amiloride. App et al. (1990) extended these studies to demonstrate that amiloride has both an acute and chronic effect on mucociliary clearance. Also, in a recent study, Davis et al. (1992) examined the potential mechanism of effect of amiloride in altering airway ion transport. Using cultured human tracheal epithelial cells, they found that amiloride (10^{-4} M) when added to the basolateral surface of the cultures inhibited the ion transport stimulatory effects of isoproterenol. Likewise, isoproterenol-induced stimulation of cAMP was inhibited by amiloride in a dose-dependent fashion. Furthermore, they showed that the binding of iodocyanopindolol, a β-adrenoceptor antagonist, was displaced by amiloride with an IC_{50} of 410 μM. They suggested that the effects of amiloride on adrenergic blockade need to be kept in mind when considering the utility of amiloride as a therapeutic agent in cystic fibrosis. Although they acknowledged a lack of response to chloride secretion secondary to β-adrenergic stimulation in cystic fibrosis, they raised the possibility that "other β-adrenergic-mediated responses (such as ciliary beat frequency)" may be adversely effected.

3. Effect of chloride channel inhibitors. A number of anthracene derivatives and related compounds, most notably diphenylamine-2-carboxylate (DiStefano et al., 1985), have been demonstrated to inhibit cellular chloride conductance. Although no studies have primarily investigated these compounds on airway secretory properties, a number have used diphenylamine-2-carboxylate to alter chloride conductance and to make inferences related to chloride conductance (Tamaoki et al., 1991; McBride et al., 1993; Schoppa et al., 1989; Stutts et al., 1988b; Smith and Welsh, 1992). For instance, Stutts et al. (1988b) showed that diphenylamine-2-carboxylate blocked chloride conductance in canine tracheal epithelium induced by increased submucosal potassium concentration. Schoppa et al. (1989), using patch-clamp techniques to measure voltage-gated chloride currents in cultured canine tracheal epithelial cells, demonstrated that diphenylamine-2-carboxylate (100 μM) suppressed these currents by 76.8%. As noted before, Smith and Welsh (1992) showed that cAMP-stimulated bicarbonate secretion across human and canine tracheal cells was decreased by diphenylamine-2-carboxylate. Suppression of this response was taken as evidence that bicarbonate exit occurs across the apical cell membrane via the chloride channel.

J. Effect of Mucolytic and Expectorant Agents on Airway Secretion

There has been surprisingly scant recent scientific interest in characterizing the mechanism of effect of mucolytics and expectorants on airway secretory processes. Many of the mucolytics in current clinical use have a thiol group which reduces disulfide bonds of mucins, rendering the mucin more fluid and thereby possibly facilitating its expectoration (Moratalla et al., 1986). For many years, iodides and iodine-containing compounds have been used as mucolytic agents. These drugs induce a bronchorrhea and thereby thin secretions (Pavia et al., 1985). Recently, a new agent, iodopropylidene glycerol, has been produced by the interaction of iodine and glycerol. This drug is now used as a mucolytic agent (Pavia et al., 1985; Petty, 1990). Representative recent studies involving both thiol group agents and iodopropylidene glycerol are described in this section.

Yanaura et al. (1982) investigated the effects of L-cysteine methyl ester on the tracheal secretory cells of dogs. Tracheas were removed from the dogs and exposed to various concentrations of L-cysteine methyl ester in vitro, and the effects of this treatment on the histological and histochemical appearance of the secretory elements of the tracheas were examined. The investigators found that low concentrations of L-cysteine methyl ester (10^{-7} and 10^{-6} M) resulted in a slight decrease in total goblet cell number. Low concentrations of L-cysteine methyl ester also resulted in a decrease in the thickness of the acini of submucosal glands and an increase in the acini inner diameter. At concentrations of L-cysteine methyl ester of 10^{-5} and 10^{-4} M, the acini appeared thicker and the acini diameters were slightly decreased, relative to control tracheas. In addition, they noted a dose-dependent relative increase in the number of goblet cells staining positively for neutral glycoproteins in response to increasing concentrations of L-cysteine methyl ester. They observed secretion into the bathing media of saccharide

and total protein at low concentrations of L-cysteine methyl ester but augmentation of this secretion at the higher concentrations. They attributed these changes to a secretogogue effect of the low concentrations of L-cysteine methyl ester and an increased synthesis of mucin at the higher concentrations. The increase in neutral glycoprotein staining suggested that L-cysteine methyl ester caused a change in the secretory product that would favor secretions of a lower viscosity product (Yanaura et al., 1982).

Konradova et al. (1985) gave rabbits a single oral dose of 100 mg of three different mucolytics: N-acetylcysteine, carbocysteine, and Na-2-mercaptoethane sulfonate. The animals' tracheal epithelia (with special attention to the goblet cells) were studied ultrastructurally 20 or 60 minutes after drug ingestion. All three agents caused alteration in the goblet cell appearance, relative to control. Twenty minutes after ingestion, evidence for goblet cell degeneration was observed, with 78% of cells degenerated with N-acetylcysteine, 49% with Na-2-mercaptoethane sulfonate, and 28.7% with carbocysteine. After 60 minutes, in the case of N-acetylcysteine ingestion, there was evidence of rapid differentiation of new goblet cells, with 86.5% of the goblet cells filled with mucus. The authors also obtained evidence that these agents caused injury to ciliated epithelial cells. They observed apical blebs in these cells following ingestion. They postulated that such changes would be accompanied by a decrement in mucociliary clearance.

Rogers and Jeffery (1986) exposed pathogen-free rats to the smoke of 25 cigarettes daily. They examined the effects of 1% N-acetylcysteine in the drinking water (average daily dose 973 mg/kg) on the thickness of the airway epithelium and the number of secretory cells in the upper trachea, mid-trachea, and at two different intrapulmonary airway levels. They observed that the thickness of the surface epithelial layer and the number of secretory cells increased in response to cigarette smoke. They noted that "prophylactic oral N-acetylcysteine led to an overall inhibition of cigarette smoke-induced mucous cell hyperplasia and epithelial hypertrophy." They suggested that N-acetylcysteine may exert an anti-inflammatory effect on airways by protecting tissues against the oxidant stress induced by cigarette smoke.

More recently, Livingstone et al. (1990) showed that purified mucus isolated from pig stomach had a reduction in the elastic modulus when exposed in vitro to acetylcysteine. Likewise, they demonstrated by gel chromatography that acetylcysteine, but not S-carboxymethlcysteine, reduced the mucous glycoprotein to smaller subunits. Studies using rat tracheal explants and examining the uptake and secretion of radiolabeled glucosamine showed that acetylcysteine (5 to 15 mM) had no effect on secretion, whereas S-carboxymethlcysteine (5 and 10 mM) reduced the production of radiolabeled mucous within 24 hours. However, utilizing mucus collected from the tracheal pouches of mini-pigs in vivo, Livingstone et al. (1990) were unable to demonstrate any significant effect of a single oral dose of either acetylcysteine or S-carboxymethlcysteine (20 mg/kg) on the rheological or biochemical properties of the mucus.

Pavia et al. (1985) studied tracheobronchial clearance in stable chronic bronchitics using inhaled radioactive polystyrene particles (5 μm diameter). In their group of 15 stable chronic bronchitic patients as a whole, they observed no statistically significant change in clearance in response to 30 mg of organically bound iodine, iodopropylidene, taken orally four times per day for 14 days. However, on an a posteriori analysis of six of these patients who were actively expectorating sputum during the time of both control and experimental observation, there was a slight acceleration of tracheobronchial clearance during treatment with iodopropylidene. The authors concluded that iodopropylidene "can be an effective expectorant in patients with mucus hypersecretion and particularly so in patients with copious sputum production."

Petty (1990) conducted a "randomized, double-blind, placebo-controlled" evaluation of the effects of iodinated glycerol, 60 mg four times per day orally for 8 weeks, versus placebo on the symptoms associated with bronchitis (cough frequency, cough severity, chest discomfort, dyspnea, and ease of bringing up sputum). The study demonstrated significant improvement in response to treatment in these variables with the exception of dyspnea. Although the global assessment by the patients of their symptoms showed significant improvement, the similar assessment by the patients' physicians did not show improvement. The detailed physiological explanation for the improvement in symptoms was only speculated on in this study.

VII. Conclusions and Future Directions

Airways are positioned at the interface of the body with the outside environment. These tissues are complex and secrete a variety of substances that serve to protect the internal milieu from the myriad of toxins and other noxious materials in ambient air. The cellular and functional complexity of airways has been known for many years.

Since the past Review (Marin, 1986), investigators have made progress in understanding the pharmacology of airway secretion on a number of fronts. They have developed new models to deal with the cellular complexity of airways; most notably, they devised techniques to culture cells from both submucosal glands and surface epithelium. This has permitted an increased appreciation of the contributions that individual cell types make to the overall secretory product.

Examination of the nervous supply of airways and the characterization of the various receptors on airway cells

have provided insights into the tremendous complexity of the physiological regulation of airway secretion. Detailed understanding of the cotransport mechanism and the operating characteristics of ion channels of the airway cells have emerged. Furthermore, the effects of environmental pollutants on airway secretion are now more fully appreciated. These agents often induce oxidant stresses that alter ion transport, permeability, permselectivity, and mucin secretion. The alterations in airway secretion in disease states (most notably cystic fibrosis) are now more fully elucidated. It is against this backdrop that further information has emerged concerning the effects of cholinergic and adrenergic drugs, active polypeptides, cAMP, intracellular calcium concentration, extracellular nucleotides, arachidonic acid metabolites, anti-inflammatory drugs, histamine, diuretics, ion channel blockers, and mucolytic drugs.

Although the elements of the various mechanisms that alter airway secretion are now more fully appreciated, it is still not entirely clear how the actions of these multiple cell types and cellular mechanisms are coordinated to perform the complex protective function of airway secretion. The techniques of molecular biology are just beginning to be applied to the problems of airway secretion. Soon, in addition to pharmacological manipulation of secretion, gene therapy may be used to reverse the abnormal secretory responses found in disease states such as cystic fibrosis (Rosenfeld et al., 1992; Rich et al., 1990; Yoshimura et al., 1992; Trapnell et al., 1991; Collins, 1992; Olsen et al., 1992).

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