Regulation of Smooth Muscle Contraction by the Epithelium: Role of Prostaglandins

As an analog to the endothelium situated next to the vascular smooth muscle, the epithelium is emerging as an important regulator of smooth muscle contraction in many vital organs/tissues by interacting with other cell types and releasing epithelium-derived factors, among which prostaglandins have been demonstrated to play a versatile role in governing smooth muscle contraction essential to the physiological and pathophysiological processes in a wide range of organ systems.

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As widely distributed throughout the body, including the blood vessels, airways, gastrointestinal, urinary, and reproductive tracts, smooth muscles have a variety of critical functions such as controlling blood pressure, respiration, and gastrointestinal peristalsis. Abnormality in smooth muscle contractility results in various disorders and diseases including hypertension, asthma, and dyspepsia (89, 95, 179). Therefore, smooth muscle contraction is tightly regulated. Apart from the long-recognized regulation by neurotransmitters released from the innervating nerve endings or hormones from the blood stream nearby (66), smooth muscle contraction has been known, for over three decades, to be regulated by the endothelium lining the blood vessels with nitric oxide (NO) as the most important endothelial-derived factor (51, 121). Over recent decades, evidence has also been accumulated indicating an important role of the epithelium, the cell layer lining the luminal surface of many organs or tracts, such as the trachea/bronchus, stomach, intestine, bladder, and reproductive organs, in the regulation of smooth muscle contraction, with a number of epithelium-derived factors identified. However, the importance of the epithelium in regulating smooth muscle tones has not been adequately appreciated. In this review, we will examine the evidence collected from a number of organ systems and hope to provide a clearer picture as to how the epithelium acts as an indispensable regulator of smooth muscle contraction, with epithelium-derived prostaglandins (PGs) as the key mediators, participating in many vital physiological processes as well as the pathogenesis of many diseases.

Indications of the Epithelium as a Regulator of Smooth Muscle Contraction

Like the endothelium lining the walls of blood vessels, the epithelium lining the lumens of all hollow organs is also in close contact with the smooth muscles surrounding the organ tubes or tracts. Similar to the endothelium facing the blood stream and thus bearing the blood pressure or the shear force from the blood flow (27), most epithelia are located at the interface of external and internal milieu and thus also bear mechanical stimulations from the lumen such as airflow, food, and fluid pressure. The epithelium is also innervated by nerve endings and expresses various receptors for neurotransmitters (104, 184). Although dysfunction of the endothelium may result in vascular diseases (e.g., hypertension) (18, 186), damage to the epithelium is also commonly accompanied by abnormal smooth muscle contraction as exemplified by the hyperresponsiveness of the airways in asthma (163). Researchers have long recognized the similarities between the endothelium and epithelium with a great interest in exploring the role of the epithelium in regulating smooth muscle contraction.

By separating the epithelium from the smooth muscle tissue in organ-bath experiments, evidence has been accumulated from several organ systems, indicating that the epithelium may influence smooth muscle contraction. As early as 1978, Whalley described a method to obtain myometrial preparations by removing the endometrial layer from rat uterus and observed that the endometrium-removed myometrial preparations exhibited decreased contractile responses to oxytoxin and bradykinin compared with the intact uterus (191). In an early study on rabbit urinary bladder, the bladder epithelium and smooth muscle strips were separated by dissection and incubated in different baths. After transferring the solution bathing the epithelium to the solution bathing the smooth muscles, the basal tension, spontaneous and electrically evoked contractile responses of the epithelium-denued smooth muscle strips were increased (47). In a later experiment on the guinea pig,
reversal of arachidonic acid-induced tracheal relaxation into contraction was observed after the removal of the epithelium (125). Studies on human bronchi and dog trachea also showed that removal of the epithelium resulted in increases in the contractions evoked by acetylcholine, histamine, or PGF2α, which could also be attenuated by subsequent addition of the medium bathing the removed epithelium (5). Another study on guinea pig uterus showed that P1-purinoceptors agonists inhibited the phenylephrine-evoked contractions of the endometrium-denuded circular myometrium but enhanced the contraction of the intact tissue (73). Later, Okpalaugo et al. demonstrated that clonidine, a direct-acting α2 adrenergic receptor agonist, relaxed the vas deferens smooth muscle in an epithelium-dependent manner (133). The differences in smooth muscle activities observed with and without the epithelium/epithelium-incubated medium reinforced the notion that the epithelium regulates smooth muscle contraction in vivo.

**PGs as a Key Epithelium-Derived Factor**

The observations that epithelium-bathing solutions could alter the contractility of smooth muscles suggested the involvement of epithelium-derived factors in mediating the relaxant or contractive actions of the epithelium. For the past few decades, several molecules have been recognized as the epithelium-derived relaxant/contractile factors, including PGs (55, 148), NO (16, 65, 159), ATP (25, 52), and endothelins (53, 74, 110). Given their well-documented effects on smooth muscle contraction and extensive involvement in many smooth muscle-mediated physiological functions (31, 48, 108, 123, 201), PGs appear to be one of the most important epithelium-derived factors.

PGs are 20-carbon chain unsaturated fatty acids synthesized from arachidonic acid, which is derived from the hydrolysis of membrane phospholipids catalyzed by phospholipase A2 (PLA2). Arachidonic acid is converted to prostaglandin G2 (PGG2) and subsequently to prostaglandin H2 (PGH2) by prostaglandin H synthase, also known as cyclooxygenase (COX) or prostaglandin-endoperoxide synthase (PTGS), which can further be divided into two subtypes, COX-1 (PTGS-1) and COX-2 (PTGS-2) (160). PGH2 is an unstable PG intermediate that is soon converted into bio-active prostanoids, prostaglandin D (PGD2), E (PGE2), F (PGF2α), I (PGI2), and thromboxane (TXA2) by their respective synthases. It is clear that epithelial cells are abundant with these PG synthases and produce PGs in vivo (77, 167). The diverse role of PGs in modulating smooth muscle tone has been extensively studied (31, 174, 201, 202). Different types of receptors specific for PGD, PGE, PGF, PGI, and TX have been identified, namely, D prostanoid (DP), E prostanoid (EP), F prostanoid (FP), I prostanoid (IP), and T prostanoid (TP) receptors, respectively (FIGURE 1). EP is further classified into four subtypes: EP1, EP2, EP3, and EP4, based on their different actions and signaling pathways activated in response to PGE2 or its analogs. The eight types or subtypes of PG receptors are found to be G-protein-coupled transmembrane proteins coded by different genes with distinctive structures. Among these receptors, activation of DP, EP2, EP4, or IP receptors increases intracellular cAMP level ([cAMP]i) and causes relaxation of smooth muscles. The EP1, FP, and TP receptors are coupled to Ca2+ mobilization and thus cause contractions. Different isoforms of EP3 have been identified, and the activation of EP3 can either increase or decrease the [cAMP]i, or increase intracellular Ca2+ but usually causes smooth muscle contractions (124). Thus epithelium-derived PGs may exert distinctive effects (contractive or relaxant) on smooth muscles in different organ systems, depending on the types of PG receptors involved (FIGURE 1).

**Airways**

PGs have long been known for their ability to regulate airway smooth muscle tone (38, 170). In particular, effects of aerosol inhalation of PGF2α and PGE2 in asthmatic patients were noted (113). However, the role of the airway epithelium as the source of prostaglandins had not been recognized until evidence was gathered on epithelium-denuded tracheal or bronchial tissues. Arachidonic acid-induced guinea pig tracheal relaxation was first found to be dependent on the epithelium and inhibited by indomethacin, the cyclooxygenase inhibitor blocking prostaglandin synthesis (125, 183). Removal of the epithelium from guinea pig trachea significantly reduced the histamine-induced release of PGE2 from the trachea and resulted in greater contractile responses to histamine (19). On rabbit bronchi (26), either removal of the epithelium or incubation of intact bronchi with indomethacin increased the sensitivity of the bronchial smooth muscle to bethanechol, a parasympathomimetic choline ester that selectively stimulates muscarinic receptors. In the same study, the PGE2 level of the medium bathing the bronchial explants was reduced after removing the epithelium, indicating the epithelium as the possible source of PGE2. These early findings suggested that histamine, arachidonic acid, or activation of muscarinic receptors triggers the release of PGE2 from airway epithelium, thereby exerting relaxant effect on the smooth muscles. Interestingly, a later study demonstrated that both histamine and arachidonic acid could stimulate PGE2 production in
the isolated trachea of guinea pig and that endotoxin-induced hyperreactivity of the trachea coincided with decreased PGE2 production by the epithelial layer (54). Subsequent studies on human airway epithelial cells confirmed their ability to produce PGE2 (30, 41, 126, 151). In clinical settings, inhalation of PGE2 was found to prevent the allergen-induced bronchoconstriction (138), whereas inhibitors of PG synthesis cause asthmatic attacks in aspirin-sensitive patients (137). These clinical observations are consistent with the laboratory findings, strongly suggesting that the airway epithelium-derived PGs are important factors modulating smooth muscle tone, defects of which could result in pathological conditions such as asthma.

Other than arachidonic acid and histamine, the release of PGE2 by airway epithelium could also be triggered by many other factors. For example, Substance P, by activating neurokinin1 receptor, was found to induce epithelium-dependent release of PGE2 and caused relaxation of rat trachea (42). On rabbit tracheal epithelium, PGE2 production was observed after stimulation of P2 purinoceptors (7). Activation of protease-activated receptor 2 by trypsin has been observed to trigger epithelial PGE2 release and relaxation of airway preparations from the mouse, rat, guinea pig, and human (32, 139). More recently, on mouse trachea, lipopolysaccharide (LPS) was demonstrated to activate epithelial Toll-like receptor 4 through the activation of COX-1 and COX-2 by nuclear factor-κB (NF-κB), leading to PGE2 production and release, and thus cause tracheal relaxation (13). Considering the diverse cell types that might produce the PG-promoting substances, these observations suggest that airway epithelial cells may respond to signals from their interacting cells, such as nerves, immune cells, and even pathogens or bacteria, with enhanced production and release of PGs, which in turn modulate airway smooth muscle activities. Interestingly, the release of PGs by airway epithelium also appears sensitive to mechanical stimuli. As early as 1975, it was reported that gentle mechanical irritation on the mucosal surface triggered the release of PGE2 and PGF2α from guinea pig trachea in vitro (135). Stretch-induced contraction was also observed on guinea pig trachea pre-relaxed by papaverine or isoproterenol, which was partially dependent on the epithelium and blocked by the inhibition of PGs synthesis (59). These observations are of importance since airway epithelia are under physiological stimuli such as the air flow and the distension during breathing. Taken together, the findings strongly indicate that the epithelium of the airways can respond to a wide range of physiological or external stimuli with the release of PGs for regulation of smooth muscle contraction.

Varied effects of PGs on airway smooth muscle activities have been observed, which is largely attributed to the expression of different PG receptors in the airway as well as their cross-talk with other neural pathways. Although most studies indicate PGE2 as an airway relaxant factor, muscle contractive effect of PGE2 has also been observed in the airways. In humans, it has been reported that inhalation of PGE2 caused bronchial dilation in healthy subjects but caused either bronchial dilation or constriction in asthmatic patients (113). Interestingly, the same study found that PGF2α reduced specific airway conductance by 50% in both healthy subjects and asthmatics, but the two groups exhibited varied sensitivities, with the patients responding to a much lower dosage. This suggested that different receptors may be expressed in pathological conditions or that components of the response pathway may be altered in patients. Using specific antagonists, it has been recognized that PGE2 exerts both a constricting and a relaxing effect on the airways by acting on
different EP receptors (60, 107, 116, 127). A study conducted by Tilley et al. (176) demonstrated that the exposure to aerosolized PGE$_2$ in conscious mice caused bronchial constrictions, which were diminished in EP$_1$- and EP$_3$-deficient mice, but not altered in EP$_2$- or EP$_4$-deficient mice. In addition, this constitutive effect of PGE$_2$ was eliminated by pre-treatment with the anti-cholinergic agent, atropine, or the anesthetic bupivacaine. In anesthetized mice or in denervated tracheal tissues, PGE$_2$ was found to cause bronchial dilations only, which were eliminated in EP$_2$-deficient mice. It is thus suggested that EP$_1$- and EP$_3$-mediated PGE$_2$-induced constriction is through an indirect neural pathway, whereas EP$_2$-mediated dilation is a direct effect on the bronchial smooth muscle cells. Interestingly airway EP$_1$-receptor was later found directly modulating $\beta_2$-adrenergic receptors on smooth muscle cells (114). Taken together, these findings suggest that the epithelium-derived PGE$_2$ (or other PGs) may exert diverse or opposing effects on airway smooth muscle cells through different receptors and cross-talk with diverse neural pathways. The involvement of cross-talk between different pathways in the epithelium-mediated regulation of smooth muscle contraction is evident by many observations. For example, it was reported that the modulating effect of the bronchial epithelium in reducing smooth muscle contraction was lost after the lung auto-transplantation in dogs where denervation or acute rejection occur (117). This observation suggests that the epithelium-dependent modulation of the smooth muscle contraction may require neural stimulation. Therefore, better treatment strategies for airway hypersensitivity or asthma patients awaited detailed study on PG receptors expression profile and their cross-talk with other neural pathways in the airways.

**Gastrointestinal (GI) Tract**

PGs are known to play a number of important roles in GI function including regulating epithelial secretion (e.g., acid, HCO$_3^-$, mucus) (37, 45, 173), mucosal cytoprotection (100) as well as the motility of the entire tract to ensure proper food intake through the esophagus, digestion in the stomach, nutrients absorption in the intestine, and bowel movement in the colon. Extensive studies have demonstrated in vitro or in vivo that PGs have influence on the contraction or relaxation of esophageal, stomach fudus, small intestine, and colon, either by acting directly on smooth muscles or interacting with the myenteric neurons (63, 97, 132). The effects of PGs on various functions of the GI tract have been extensively reviewed elsewhere (43, 48) and will not be covered here.

The involvement of the mucosal epithelium in regulating PG-dependent processes in the GI tract is evident by the fact that PGs are produced throughout the entire GI tract with the mucosa layer as the major source (33, 146). COX-1, as the predominant PGs synthase in the GI tract, is expressed in mucosal epithelium (143). Surprisingly, despite the mounting evidence implicating a role of the epithelium in regulating GI functions through PGs production (33), no direct evidence has been documented, demonstrating the involvement of the epithelium of regulating GI motility. However, some studies have indicated the possibility. For instance, it has been observed in vitro that activation of protease-activated receptors caused increased release of PGs from human intestinal epithelial cells (24) and rat gastric epithelial cells (157, 180), whereas PG-dependent contractions induced by the activation of protease-activated receptors have also been observed in rat and mouse stomach (149, 156). In addition, LPS, the major component of the outer membrane of bacteria, has also been found to induce PG-dependent intestinal contraction and epithelial release of PGs in the rabbit (145). Since the mucosal epithelium, but not the smooth muscle, is in direct contact with luminal substances, the involvement of the epithelium in mediating the signals from the lumen to the smooth muscle is inevitable. The observations that luminal substances, such as proteases and bacterial LPS, can stimulate both epithelial PG production/release and smooth muscle contractions strongly indicate an important role of mucosal epithelium in regulating GI motility in response to stimuli present in the GI tract lumen.

PGs have also been proposed to be involved in cytoprotection of the stomach. Notably, nonselective NSAIDs induce gastric injury with both a marked decrease in PGs level in the gastric mucosa (185, 192) and a significant increase in the amplitude and frequency of stomach contractions or gastric hypermotility (172). By working on rat models, it has been demonstrated that PGE$_2$ is continuously produced by gastric mucosa and maintains a normal motility in the stomach, whereas disruption of mucosal PGE$_2$ synthesis by NSAID may result in gastric hypermotility, which causes temporal restriction of blood flow, hypoxia, and thus damage to the stomach (171, 172). Importantly, the mucosa-generated PGs are known to decrease acid production, promote epithelial secretion of HCO$_3^-$ and mucus, increase blood flow, and facilitate epithelial cell renewal in the stomach (98). Thus, together with their action in regulating motility, the mucosa-derived PGs are believed to be crucially involved in gastric cytoprotection and mucosal defense to noxious factors (e.g., HCl and pepsin) in the lumen. Along this line, the involvement of mucosal epithelium in mucosal defense is further suggested by the observed protective effect
of melatonin against GI damage or ulcers (83) and for improving GI motor dysfunction such as gastroesophageal reflux disease (190) and irritable bowel syndrome (105). Although originally known to be secreted by the pineal gland, melatonin can also be produced and secreted by serotonin-rich enterochromaffin cells of the GI mucosa and act as an autocrine and paracrine hormone. Melatonin appears to exert its effect on GI motor function by antagonizing the effect of serotonin by interacting with serotonin receptors on the vagal afferent fibers (175). Melatonin may also be involved in PG production by interacting with vascular endothelium and sensory nerves (83) to regulate GI motility and exert its protective effect. Further work along this line may help to elucidate the detailed mechanism by which melatonin exerts its protective effect on GI motor function.

Motility disorders of the GI tract, with either increased or decreased smooth muscle contractions, are frequently associated with intestinal inflammation, notably as in inflammatory bowel disease (131). The disturbance of the motility has been largely attributed to either smooth muscle dysfunction or changes in the enteric nervous system under the influence of immune cells (131). Surprisingly, the epithelium, which is innervated and in close contact with immune cells and the smooth muscle, has received very little attention for its possible role in regulating smooth muscle tones during inflammation, although its role in coordinating inflammatory responses and PG production during inflammation has been recognized (33). However, several lines of evidence do suggest the contribution of the mucosal epithelium to GI smooth muscle tone during inflammation. Colonic ulceration is known to be associated with inflammatory bowel disease (49). In mice, acute colitis induced by oil of mustard, with inflammatory damage and loss of the epithelium, has been shown to develop inflammatory bowel syndrome-like changes in motility (92). Interestingly, long-term functional changes in motility were observed after the acute and transient colitis when there was no gross inflammation. The observed colonic ulceration and altered motility in this animal model suggests that the loss or damage of epithelium might contribute to the motility abnormality in intestinal inflammation disease. Of note, increase in tissue concentrations of PGs has also been observed in patients with ulcerative colitis (144, 193). The conversion of arachidonic acid to its metabolites in the mucosa of patients with inflammatory bowel disease was also found to be significantly higher compared with that of healthy subjects (136). Taken together, it appears that the mucosal epithelium may play a significant role in influencing GI motility in inflammation. Further supporting this notion, a recent study has demonstrated abnormal morphology or damage to colonic epithelium in rats with enhanced stress-induced anxiety (128). Since stress is known to affect epithelial barrier function and immune response (99), PG production by GI mucosa (165), and GI motility alterations, including inhibition of gastric emptying, stimulation of colonic propulsive motility, and hypersensitivity to colorectal distension (166), the observed damage to mucosal epithelium in stressed animals further indicates its possible involvement in gut motor dysfunction during inflammation; however, the detailed mechanisms require further investigation.

**Urinary Tract**

The role of PGs in urinary smooth muscle contraction and micturition (urination) reflex, the relaxation of the urethral sphincter in response to increased pressure in the bladder, has been extensively studied ever since the first finding in 1971 showing that prostaglandin-like material was released during or right after bladder distension in dogs (62). It was soon recognized that PGs regulate the smooth muscle contraction (2, 23) as well as modulate the neural transmission in the urinary tract (40, 87, 108). Meanwhile, PG production was observed in the bladder of human (3) and other species (21, 85, 142). Similar to the airways, the release of PGs by the bladder could be stimulated by various factors, including neuropeptides (e.g., substance P, neurokinin A) (109) and inflammation factors (e.g., bradykinin) (141), which cause urinary bladder contraction and facilitate the micturition reflex. Changes in pH, osmolarity, or distension of the bladder were also found to alter PG production by rat bladder (85). These findings suggest that the PG production by the bladder, and thus the contractility of the bladder, may be altered in response to a variety of neural and physiological stimuli.

The role of urothelium, or the epithelial layer of the urinary bladder, in PG production was first demonstrated in the rabbit by thin-layer chromatographic analysis and radioimmunoassay, which appeared to be distinctively different from that produced by the outer vesicular layer of the bladder (21). In the study conducted by Downie and Karwazyn (47), the solution pre-incubated with the epithelium contracted the detrusor smooth muscle in rabbits, which could be reduced by treating the epithelium but not the muscle with indomethacin and ibuprofen, inhibitors of COX. In the same experiment, addition of several PGs contracted the smooth muscle, mimicking the effect of the epithelium bathing solution. These results suggest that the epithelium-derived PGs can regulate the contractility of urinary bladder. PG release by human urothelium was also confirmed (86), reinforcing the
PGE2 production may be the cause of OAB. Indeed, overactivity (81, 154), indicating that abnormal PGE2 into bladders of rats and mice caused bladder der (OAB) syndrome. Intravesicular infusion of detrusor overactivity (DO) and the overactive bladder is believed to underlie urinary diseases like the function of urinary bladder.

urothelium-derived PGs in maintaining the normal observations further indicate an important role of the present with bladder overactivity (112). These observations are consistent with the notion that aberrant PG levels affect spermatogenesis. Indeed, higher PGs levels responded to indomethacin treatment with a significant increase in sperm count and motility (34, 122), suggesting that aberrant PG levels affect spermatogenesis. Indeed, although COX-2 protein is not normally detected in human testis (71, 129), abnormal expression of COX-2 has been found to be associated with impaired spermatogenesis in men (58, 119). However, the exact mechanism is not entirely clear, although a most recent study (152) has linked male sub-/infertility to PG metabolite-induced hypertrophy and loss of contractility in human testicular peritubular cells, the smooth-muscle-like cells in the seminiferous tubules.

In mammals, there are two major mechanisms for the mammalian testes to propel the immotile sperm in the seminiferous tubules through the rete testis and the epididymis. First, the seminiferous tubules produce the so-called PGs to trigger seminiferous tubule contraction. An early study (36) examining the potential role of PGs in the regulation of the contractions of the testicular capsule and the seminiferous tubules (35). Interestingly, the PG production in rats was also found to be sensitive to FSH, the gonadotropin controlling spermatogenesis, indicating the involvement of Sertoli cell-derived PGs in sperm production in the testis (35, 82). The expression of COX2 in the testis has been found in Leydig cells, Sertoli cells, and spermatogenic cells of the rat (80, 198), but only in Leydig cells of the hamster (57). The exact roles of PGs from different cell types, particularly those from Sertoli cells, in spermatogenesis remain largely unknown. Since Sertoli cells are known to actively secrete electrolyte and fluid for transporting immotile sperm out of the testis (84), it is tempting to speculate that they might also produce PGs to trigger seminiferous tubule contraction to propel the immotile sperm along with the fluid secreted.

After leaving the testis, immotile sperm gain their motility during their transit through the head (caput) region of the epididymis (204) and undergo further maturation process (200) until they reach the cauda (tail) epididymis where they are stored in a quiescent state until ejaculation, on which they are transported to the urethra by vas deferens contraction. An early study (36) examining the
effects of PGF$_{2\alpha}$ and PGE$_2$ on rat caput epididymidis contractility demonstrated that PGF$_{2\alpha}$ stimulated the contractility, whereas PGE$_2$ reduced it. Endogenous levels of the two PGs were also measured in both rat (36) and mouse (11) epididymis. A more recent study on rats (164) examined the localization of COX-2 in the male reproductive tract and found that COX-2 was expressed in the initial segment and caput epididymidis but not in cauda epididymis. These observations are consistent with the role of initial segment and caput epididymis, where smooth muscle contraction is required for sperm transport, and the role of cauda epididymis for sperm storage, where sperm are kept in a quiescent state with minimal contractile activity anticipated. The role of the epithelium on epididymal contraction was clearly conducted by Mewe et al. (120), in which spontaneous contractions (SCs) of bovine epididymis were shown to be completely blocked by the removal of the epithelium. PGF$_{2\alpha}$ receptor antagonist reduced the SCs of intact epididymal tubes, whereas PGF$_{2\alpha}$ induced SCs-like responses on epithelium-denuded tissues. These results strongly indicate the involvement of the epithelium and its PGs in the regulation of epididymal contractility.

Being a muscular tube, the vas deferens is generally considered as a conduit to move sperm from the epididymis to the urethra on ejaculation. Early studies detected high levels of PGs in the vas deferens of the mouse and rat (11, 36), which could be altered during sexual maturation or by changes in testosterone levels (11, 12), suggesting a possible role of PGs in controlling sperm transit through the vas deferens on sexual maturation. Indeed, the effects of both PGF$_{2\alpha}$ and PGE$_1$ in altering the contractile response of rat vas deferens to adrenergic stimulation were subsequently observed (28). A study examining the effects of PGs of the D, E, F, and I series on neuromuscular contraction of rabbit vas deferens showed that all PGs tested could inhibit neuromuscular contractions but with varied effects on contractile responses to different neuromuscular stimulation, such as adrenergic, nonadrenergic, and ATP (purinergic) (181). The effects of PGs on electrically induced contractions of the vas deferens were also observed in the guinea pig (29) and the human (76). Despite the mounting evidence indicating an important role of PGs in modulating vas deferens contractions, either neuromuscular or electrically induced, the exact source of endogenous PGs remained unclear. Interestingly, a study conducted by Okpalaugo and colleagues (133) demonstrated that the removal of rat vas deferens epithelium could alter the contractile response of the rat vas deferens to adrenergic stimulation, indicating the influence of the epithelium on vas deferens contractility. Furthermore, an immunohistochemical study (94) has localized COX-2 to the epithelial cells of the ejaculatory ducts, which consists of both the vas deferens and seminal vesicles, suggesting that the epithelium along the male reproductive tract may be a possible source of PGs. We have undertaken a recent study to elucidate the detailed mechanism by which the epithelium affects vas deferens contractility (148). In that study, we observed that ATP induced an epithelium-dependent and indomethacin-sensitive inhibition of vas deferens contraction. In isolated epithelial cells and smooth muscle cells, ATP was found to promote PGE$_2$ release from the epithelial cells, but not smooth muscle cells, through P2Y purinergic receptor-coupled Ca$^{2+}$ mobilization. In the isolated smooth muscle cells, ATP could cause a lasting depolarization favoring muscle contraction, which could be reversed by subsequent application of PGE$_2$, with the activation of cAMP-dependent K$^+$ channels resulting in membrane hyperpolarization that would relax the muscle. This study has provided for the first time the details how the nerve, epithelium, and smooth muscle in the vas deferens may interact to fine-tune the contractility of the vas deferens in response to purinergic stimulation. As depicted in FIGURE 2, ATP, after leaving the nerve ending, directly induces contraction of the smooth muscle cells and at the same time triggers the release of PGE$_2$ from the epithelial cells, which in turn acts on the smooth muscle cells to trigger the signaling events leading to hyperpolarization and relaxation of the vas deferens. In light of the versatile role of purinergic receptors in control of lower genitourinary tract function, their potential as targets for the development of non-steroidal male contraceptives has been proposed (64). Along this line, our findings have shed new light on the understanding of the detailed mechanism underlying purinergic control of sperm delivery through the vas deferens involving epithelium-derived PGE$_2$ (155). Together with the findings that the COX-2 expression in rat vas deferens epithelium is androgen-sensitive (115) and the observations that PGs may promote vas deferens epithelial anion secretion (likely HCO$_3^-$) in the swine and human (140), which could stimulate sperm motility during ejaculation, the demonstrated involvement of epithelium-derived PGE$_2$ in regulating vas deferens contraction has provided compelling evidence indicating that the vas deferens is much more than just a muscular tube but a complex functional tissue with multiple players contributing to the successful delivery of viable sperm during ejaculation.

Most recently, EP$_2$-mediated inhibition of smooth muscle contractility has also been observed in rat prostate gland (178). Interestingly, stimulation of cannabinoid receptor 1 that is exclusively expressed...
in rat prostatic epithelium has also been found to inhibit prostatic contraction, which could be mimicked by PGE₂ and significantly reversed by indomethacin (177). It seems likely that a mechanism involving the epithelium and PGE₂ may also be present in the prostate to control its contractility. However, the expression of COX-1 and COX-2 in the epithelium of normal prostate has not been demonstrated convincingly, although high levels of their expression were found in prostate cancer (93, 94).

**Female Reproductive Tract**

It has been extensively documented that PGs participate in a series of reproductive events in the female reproductive tracts with their major role in modulating smooth muscle contractility. In the uterus, the endometrium, the epithelial layer of the uterus, is believed to be the major source of PGs (195, 196). The fact that shedding off the endometrium during menstruation results in increased contraction of the myometrium, the smooth muscle layer of the uterus, with severe cases exhibiting menstrual cramps or dysmenorrhea, pains in the abdominal and pelvic areas caused by excessive uterine contraction, indicates that the normal muscle tone of the uterus is under the influence of the endometrium. It has also been found that the PG levels in the endometrium in patients with dysmenorrhea are higher than those observed in normal women (106). These clinical observations indicate the possible involvement of the endometrium-derived PGs in the regulation of uterine smooth muscle contraction.

PGs are indispensable to the coordinated contraction/relaxation of the myometrium, especially during gestation and parturition since inhibition of PGs synthesis can prolong labor in rats (4). The expression of contractile PG receptors, FP, EP₁, and EP₃, has been found to be decreased in the myometrium during human pregnancy but increased during labor (20, 46). However, the relaxatory EP₂ and IP receptor-mediated responses are found to be increased in human pregnant myometrium than in non-pregnant ones (158), with increased EP₂ expression found in pregnant human myometrium (20). Thus the decrease in the expression of contractile PG receptors with increase in relaxant receptor expression results in a predominant relaxatory effect of PGs, which may ensure the uterus in a quiescent state during pregnancy. Importantly, a study on baboon uterus revealed that, during labor, the expression of EP₁ and EP₃ receptors was higher in the uterine fundus compared to the cervix and corpus (20).
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with that of lower segments, whereas EP₂ expression was lower in the fundus (161). Thus the spatially differential expression of PG receptors along the uterine tract may contribute to the contraction of the myometrium in the upper part and the dilation of cervical/lower part of the uterus observed during labor, which is believed to be essential to expel the fetus during parturition.

Although PGs were observed to be present at the myometrium, decidua, and amnion membrane in women during labor (197), several studies have demonstrated that the endometrial epithelial cells could be the source of PGs essential to parturition. Using in situ hybridization, the COX-1 expression in mouse uterus on the day of delivery was found to be restricted to uterine epithelium encircling the fetus and the luminal epithelium of interimplantation region (147). Oxytocin, the important neuromodulator in labor, has been demonstrated to regulate COX-2 expression and induce PGs release from the endometrium (9, 17, 191). In addition, in human cervix during labor, COX-2 expression was observed predominately in the endocervical glandular epithelial cells, which is believed to contribute to the PGE₂ production and cervical dilation observed during labor (72).

Notably, disorder of PGE₂ production resulting in early dilation of the cervix is believed to be associated with preterm labor, including infection-induced preterm labor. Interestingly, inhibitors of PGs synthase could prevent preterm labor (134). With most studies focusing on the PG synthesis on myometrial cells during infection, less attention has been paid to the epithelium, which, as mentioned above, is the major source of PGs in laboring cervix (72) and in close contact with bacteria when infection occurs. In addition, LPS has been shown to induce release of PGE₂ from the endometrium (75), and toll-like receptors have been found in the endometrial epithelial cells (39). Taken together, these results suggest that abnormal production/release of the endometrium-derived PGs may be one of the underlying causes of preterm labor.

In the oviduct, several PGs haven been identified to be involved in controlling the activities of oviductal smooth muscle, which are necessary for propelling sperm to the ampulla for fertilization or the embryo to the implantation site in the uterus (101–103, 189, 194). Oviductal epithelium is also a major site for PG production since PG activities have been found in the endosalpinx, the epithelium of human fallopian tubes (130). More recently, COX-1, COX-2, PGIS, and PGES have also been found in human or bovine oviductal epithelium (61, 79). The possible involvement of the endosalpinx in regulating oviduct contraction is suggested by an interesting observation that, after scraping off the endosalpinx, the frequency and amplitude of the spontaneous contractions of the ampulla of rabbit oviduct were altered, although still exhibited spontaneous contraction and electrical field stimulation-evoked and phenylephrine-induced contractions (70). However, more thorough demonstration of the role of epithelium-derived PGs in regulating oviduct contraction awaits further investigation.

In the mammary system, PGs have been considered to be involved in the process of lactation. PGF₂α or PGE₂ has been reported to modulate the effect of oxytocin and thus milk ejection in lactating animals and women (44, 187, 188). Also, in lactating cows, sows, rabbits, guinea pigs, and rats, a direct action of PGs on mammary myoepithelial smooth muscle contractility is usually accompanied by milk-stasis, it remains an open possibility that abnormal epithelium-derived PGs and thus overcontraction of the myoepithelium might be the underlying cause.

**Summary and Future Directions**

Although only limited information is available in the literature, we are still able to see an emerging role of the epithelium as an important regulator of smooth muscle contraction with its own production of PGs as one of the key mediators. With its wide distribution in many vital organs and systems, PGs have been found to transduce stimuli to produce and release PGs. This emerging role of the epithelium has shed new light on our understanding of the integrative physiology of various organ systems and should open up new avenues of research and opportunities.

**Cross-Talk Signaling Pathways**

As in blood vessels, where balanced vascular smooth muscle contractility is usually a result of the interaction between the muscle, nerve, and endothelium involving neurotransmitters and endothelium-derived factors, the epithelial layers lining many organs and tracts may produce and release PGs, as well as other factors such as NO and endothelin that are not reviewed here, in response.
to different physiological stimuli, such as histamine, acetylcholine, oxytocin, and bradykinin, which may have originated from the nerve, blood stream, or immune cells, and then in turn regulate smooth muscle contraction. However, the sequence of events involved or the detailed signaling mechanisms underlying many of the cross-talks are not fully elucidated. Particularly, the signaling events leading to the release of PGs by the epithelium in a physiological context remain vastly unknown. In addition, the knowledge about the expression regulation of PG synthases and receptors under various physiological conditions is far from adequate. The spatially and temporally differential expression patterns of PG receptors, as seen during pregnancy and at the term of labor, as well as the timely release of PGs on stimulation (e.g., by purinergic ATP), would be the key to coordinated interaction between different cell types for fine tuning the smooth muscle contraction required for a complex physiological task, such as parturition or sperm transport through the vas deferens on ejaculation.

**Luminal Sensor and Physiological Regulator**

Instead of facing the blood stream as the endothelium, the epithelium is facing the external environment and encountering a variety of substances and cell types, including germ cells and bacteria that pass through the lumen. The epithelium does not merely act as a physical barrier but also serves as a sensor to the dynamically changing external environment and a regulator or coordinator of different physiological events, such as secretion, absorption, and smooth muscle contraction, to protect and ensure proper functions of different organs. For example, as the first line of defense, the epithelium protects against pathogens or bacteria by transducing the signals from irritants or pathogens, such as LPS, into a cascade of signaling events leading to the

**FIGURE. 3. Regulation of smooth muscle contraction by the epithelium with PGs as key mediators**

The hypothetical model shows that the epithelium receives signals from nerve endings, the blood stream, immune cells (e.g., macrophage, lymphocyte, neutrophile), germ cells (sperm, blastocyst), and luminal factors (e.g., mechanical stimuli, pathogens, pH, CO₂), which can trigger PGs release from the epithelial cells and in turn regulate smooth muscle contraction.
release of PGs, which may, on the one hand, stimulate electrolyte and fluid secretion to flush out the irritant or pathogen and, on the other hand, increase the contractility of the smooth muscle to propel the pathogens. This scenario can be readily seen in the airways and GI tract. The upper GI mucosa is also exposed to different chemicals, such as gastric acid, CO₂, and ingested foods, which may be harmful to the mucosa. Luminal chemosensing by the mucosa has been considered as one of the most important mucosal defense mechanisms in the GI tract, which also involves COX pathways (6). However, how GI motility is affected by various chemicals, such as H⁺ and CO₂, and the details of the chemosensing mechanisms and downstream pathways require further studies.

In the reproductive tract lumen, transiting sperm, egg, or embryo/blastocyst may release ATP or proteases (118, 150), which have been clearly demonstrated to trigger the release of PGs from epithelial cells (7, 32, 148). Therefore, the coordinated PGs release and fine-tuned smooth muscle contraction on the interaction of the epithelium with germ cells are all necessary for successful reproduction. However, knowledge is still lacking of the types of signals released from the germ cells or embryo at different times and in different segments of the reproductive tracts. Detailed understanding of the signaling events involved may reveal potential defects that may underlie infertility or provide molecular targets for the development of effective contraceptives.

As demonstrated in the urinary bladder and airways, the release of PGs by the epithelium is sensitive to mechanical stimulation (47, 111, 112, 135). This is of particular interest since many epithelial cells are exposed to dynamically changing environment and thus constantly under mechanical stimulations, such as the air flow in the airways and the increased pressure after food filling in the stomach and GI tract. Interestingly, epithelial cells are also known to express mechanical-sensitive ion channels, such as transient receptor potential channel (TRP) (96) and epithelial sodium channel (ENaC) (56), which may serve as signal transducers. Of note, our recent study has discovered that activation of ENaC could lead to PGE₂ release from the endometrium, which may be involved in the process of implantation. Most recently, the mechanosensitive gating property of the cystic fibrosis transmembrane conductance regulator (CFTR) has been identified (203). This would have far-reaching implication since CFTR is expressed in a wide variety of epithelial cells lining different organ systems. These new findings may lead to future work providing new insight into how smooth muscle activities may be modulated by external forces or mechanical stimuli.

Pathogenesis and Treatment Strategies

Abnormal PG production or disrupted signaling cascade leading to PG release by the epithelium has been recognized as one of the important causes underlying many disease processes with smooth muscle disorders, such as asthma, overactive bladder, dyspepsia, and dysmenorrhea. Other disease processes, such as irritable bowel syndrome or inflammatory bowel disease and infertility, might also be attributed to the defects related to epithelium-derived PGs, but the detailed mechanisms are yet to be elucidated. Treatment strategies aiming either to provide exogenous source of PGs or to suppress the endogenous production of PGs have derived with aerosols of PGs or indomethacin. In fact, inhalation of PGE₂ by patients with asthma has been observed to cause marked inhibition of the early and late response to allergen (138). Since cellular responses to PGs depend largely on the types of PG receptors, recent treatment strategies have been proposed to selectively target specific PG receptors (8, 88, 153, 201). Obviously, detailed knowledge on the types of PG receptors involved in mediating smooth muscle contraction and their expression in health and disease states would help toward devising new treatment methods for related smooth muscle disorders.

In closing, evidence accumulated over the last several decades has demonstrated the ability of the epithelium to regulate smooth muscle activities, with PGs as key mediators of its action. Further research along this line should enhance our understanding of many vital physiological and pathophysiological processes and may provide grounds for the development of new treatment strategies.

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