EXPERIMENTAL ESOPHAGITIS AND SIGNAL TRANSDUCTION TO SMOOTH MUSCLE MOTILITY

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BACKGROUND

Lower esophageal sphincter (LES) is characterized by the ability to maintain a sustained pressure, and to relax allowing the passage of a bolus, whereas the esophagus is normally relaxed and contracts only briefly when required to produce peristalsis (fig. 1). The neuromuscular mechanisms that participate in the physiological regulation of these functions are not well understood, but it is thought that LES tone is spontaneous and regulated mostly through myogenic mechanisms, whereas LES relaxation and esophageal contraction are induced by neural mechanisms.

Gastroesophageal reflux represents the effortless movement of gastric contents from stomach to esophagus. Because this phenomenon occurs in virtually everyone multiple times every day and in the majority of people without clinical consequences, the reflux per se is not disease. However in some cases, it can be pathologic, producing symptoms and signs called gastroesophageal reflux disease (GERD), which mechanism is not well known. It may result in heart burn, chronic esophagitis, aspiration pneumonia, esophageal strictures, and Barrett's esophagus.

Esophagitis is a multifactorial disease that may depend on inappropriate LES relaxation, speed of esophageal clearance, mucosal resistance and other factors and is often associated with low LES pressure. Inappropriate relaxation of the LES during sleep may induce reflux of acid into the esophagus. Sleeping subjects swallow infrequently and, unless secondary peristalsis is promptly evoked by the reflux's episode, acid may remain in the esophagus for a prolonged period (Dodds et al, 1982; Helms et al., 1983, 1984).

Potency of the refluxate: The substances found in stomach that can contribute to the noxious quality of the refluxate include: HCl, pepsin, bile salts, and panceatic enzymes(tripsin, lipase). At acid pH and hydrogen ions (H^+) are the major injurious agents in the refluxate, and their capacity for injury to the esophageal epithelium is concentration and time dependent. Subjects with reflux esophagitis have been reported to secrete gastric acid at rates equal to or greater than healthy subjects (Colman et al., 1984). In either case, the pH of the refluxate can be less than 1, a value readily able to produce esophageal damage. Three components that are antireflux barriers, luminal clearance mechanism, tissue resistance can protect against noxious gastric contents.

Treatment: Antacids, H_2 blockers, H^+ pump inhibitor (healing rate over 90 %) and prokinetic agents are used for improvement of these symptoms. Irritable foods are not recommended. As one of the major acid-peptic disease, reflux esophagitis is in part responsible for the expenditure worldwide of 2 to 3 billion dollars per year for anti-ulcer prescription and nonprescription drugs.

Experimental Model: Biancani et al. (1984, 1992) has previously shown that cat acute experimental esophagitis (caused by 0.1N-HCl perfusion, 45ml /45 min for 3-4 days) affects contraction motility in the body of the esophagus and in the LES in vivo and mimics some of the symptoms found in patients with GERD, namely reduction in amplitude of esophageal contraction and reduction in resting LES pressure.

AIM AND APPROACH

In the current cycle we have investigated myogenic mechanisms mediating LES tone and esophageal contraction, and we have made substantial progress towards understanding these mechanisms and the damage produced by acid-induced inflammation. We propose to follow these leads. ACh is an important neurotransmitter in the esophagus, since it is at least in part responsible for muscle contraction during esophageal peristalsis. The strength of the proposal derives from an integrated approach, involving pharmacological, physiologic and biochemical techniques in whole tissues and in single smooth muscle cells, and focuses on the investigation of the factors responsible for contraction of esophageal and LES smooth muscle.

We found that LES and esophageal signalling to muscle contraction are different and that these signalling pathways are affected by acid-induced damage in a model of experimental esophagitis.

SUMMARY AND DISCUSSION

1. ESOPHAGEAL CONTRACTION IN RESPONSE TO ACH (fig.)
2. LES CONTRACTION IN RESPONSE TO ACH (fig.)

3. ESOPHAGITIS

Acute experimental esophagitis affects contraction in the body of the esophagus and in the LES and mimics the low amplitude of esophageal contraction and low resting LES pressure found in some patients with chronic esophagitis. The response of circular muscle from the body of the esophagus to elevated KCl (Biancani et al., 1984) or ACh (Biancani et al., 1992) is not affected, suggesting that esophageal circular muscle function is not damaged. However, the in vivo response to swallowing and the in vitro response to electrical (i.e., neural) stimulation is reduced, suggesting impairment of the neural mechanisms responsible for release of excitatory neurotransmitters.

In circular muscle from the LES the magnitude of contraction in response to maximally effective doses of ACh is not affected by acid perfusion. However, the intracellular pathways utilized for contraction are different from those utilized in the normal LES. Preliminary data suggest that basal IP_3 levels (Biancani et al., 1992) and intracellular Ca^{++} stores may be significantly decreased. These data may explain the reduction of LES basal tone observed after induction of experimental esophagitis. In addition the pathway utilized for ACh-induced contraction switches from one dependent on release of intracellular Ca^{++} and activation of calmodulin, to one that depends on influx of extra-cellular Ca^{++} and activation of PKC (Rich et al, 1997). It is possible that depletion of intracellular Ca^{++} stores caused by acid-induced inflammation may prevent Ca^{++} storage and/or release and calmodulin activation, and may somehow cause a switch to a pathway dependent on Ca^{++} influx and PKC activation.

(1) Effect of esophagitis on esophageal contraction

In the cat esophageal contraction is mediated by ACh since in the normal esophagus contraction in response to electrical (i.e. neural) stimulation is antagonized by atropine (Behar et al.1989). After induction of experimental esophagitis by repeated acid perfusion of the esophageal lumen, the response of esophageal strips to KCl (Biancani et al., 1984), and the response of strips and single cells to ACh (Biancani et al, 1992) are not affected. However contraction in response to in vivo swallowing or in vitro electrical stimulation is significantly reduced. These data suggest that esophageal smooth muscle is not damaged by acid

perfusion, but that the neural mechanisms responsible for release of excitatory neurotransmitters may be affected. Pilot studies suggest that after induction of experimental esophagitis lower doses of atropine are required to antagonize contraction in response to electrical stimulation, and this is consistent with reduced ACh release in response to electrical stimulation.

We found that esophagitis increased the role of secretory PLA_2 in contraction of esophageal circular muscle. The PLA_2 antagonists dimethyleicosadienoic acid and AA-COCF3 (trifluoromethylketone analog of arachidonic acid) inhibited ACh-induced contraction of ESO in normal and esophagitis' animals, suggesting that arachidonic acid formation contributes to ACh induced contraction both in normal and esophagitis animals. Cyclooxygenase and lipoxygenase metabolize arachidonic acid into prostaglandins and leukotrienes (LT) respectively. The cyclooxygenase inhibitor indomethacin slightly reduced ACh-induced contraction of normal esophageal muscle. This inhibition was slightly increased after esophagitis. In contrast, the lipoxygenase inhibitor nordihydroguaiaretic acid had no effect on ACh-induced contraction of normal esophageal muscle, but caused almost complete inhibition of contraction after esophagitis. The leukotriene D_4 (LTD_4) receptor antagonist ICI 198,615 had no effect on ACh-induced contraction of normal esophagus but dose-dependently inhibited esophageal muscle in esophagitis animals, resulting in complete inhibition at 10^{-7}M. LTD4 may be converted to leukotriene E_4 (LTE_4) by glycinase. The glycinase inhibitor L-cysteine did not alter the inhibitory effect of ICI 198,615, indicating that LTE4 may not contribute to ACh induced contraction (Kim et al., 1997).

These data suggest that unlike normal esophageal muscle, where arachidonic acid but not its metabolites play a role in ACh-induced contraction, LTD_4 may participate in ACh-induced contraction of esophageal circular smooth muscle cells after acid induced inflammation.

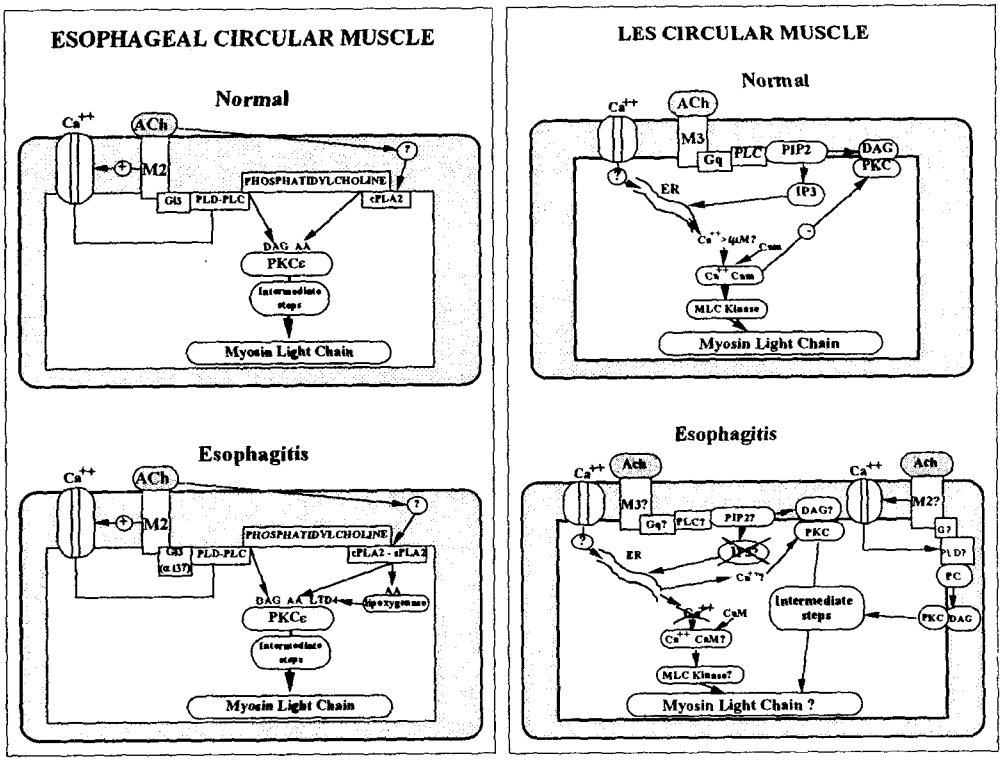
(2) Effect of esophagitis on contraction of LES muscle (fig.)

In the LES experimental esophagitis affects the mechanisms responsible for maintenance of tone and for contraction in response to ACh. In vivo and in vitro lower esophageal sphincter tone is reduced more than 80 % and reduced resting IP_3 levels more than 90%, and a switch occurs in the intracellular pathways responsible for LES contraction in response to ACh, suggesting that the mechanisms responsible for spontaneous production of IP_3 may be damaged. After induction of esophagitis, contraction is also no longer mediated through a calmodulin-dependent pathway. It is mediated through PKC-dependent mechanisms, since it is blocked by PKC antagonists and not affected by calmodulin antagonists. The possibility that the mechanisms responsible for Ca^{++} storage or uptake may also be damaged has not been explored.

(A) Affect Ca^{++} store: In the present investigation we examined the response of LES muscle cells to IP_3 and to thapsigargin in normal animals and in cats with experimental esophagitis. IP_3 releases intracellular Ca^{++} by binding to specific receptors in the endoplasmic reticulum. Thapsigargin causes release of IP_3 sensitive and insensitive intracellular Ca^{++} stores and inhibits ATP-dependent Ca^{++} uptake. In single cells isolated by enzymatic digestion from LES circular muscle and permeabilized by saponin, contraction in response to IP_3 was reduced by approximately 50% after induction of experimental esophagitis. Maximal contraction in response to 3 μM thapsigargin was reduced by approximately the same amount. In contrast the response to the protein kinase C agonist 1,2- dioctanoylglycerol (DAG) was not affected. Additionally, contraction of permeable LES

cells in response to calmodulin, in the presence of 1.3-3.3 μM cytosolic Ca^{++} levels, was the same in control animals and in animals with experimental esophagitis. We have previously shown that these high cytosolic calcium levels are required to support calmodulin induced contraction. These data show that in LES circular muscle experimental esophagitis inhibits contraction induced by IP_3 and thapsigargin, which release calcium from intracellular stores. Experimental esophagitis does not affect LES contraction in response to DAG, which activates PKC, or in response to calmodulin, which in the presence of Ca^{++} activates MLCK. The data support the hypothesis that experimental esophagitis causes damage to intracellular calcium stores but does not affect contraction mediated by direct activation of protein kinase C or of MLCK.

(B) A second pathway mediated by M_2 receptors linked to G_{i3}, PC-PLC and -PLD may be activated: We examined that in normal LES cells contraction is mediated by M3 receptors linked to $G_{q/11}$ and PI-PLC, while after esophagitis, contraction through this pathway is reduced since IP_3 production and Ca^{++} stores are damaged. In normal LES cells maximal contraction in response to ACh is inhibited by the M_3 antagonist p-flouro-hexahydro-sila-difenidol (pF-HSD), and not by M_1 or M_2 antagonists. In AE cells contraction was inhibited by the M_2 antagonist methoctramine and by pF-HSD, suggesting that ACh activates both M_2 and M_3 receptors. We examined the G-proteins linked to these receptors by using specific G-protein antibodies in cells permeabilized by brief exposure to saponin. ACh induced contraction was antagonized by antibodies against $G_{q/11}$ in normal cells, and by $G_{q/11}$ and G_{i3} antibodies in AE. In addition ACh induced contraction of normal LES was reduced by the phosphatidylinositol specific phospholipase C (PI-PLC) antagonist U 73122 (10^{-6}M), but not by the phosphatidylcholine specific phospholipase C (PC-PLC) inhibitor D609, or by the phospholipase D (PLD) pathway inhibitor propranolol. In



esophagitis contraction was reduced by U73122, by propranolol and by D609, suggesting that contraction in response to ACh in esophagitis may be mediated through both $G_{q/11}$ protein activating PI-PLC and G_{i3} protein activating PC-PLC or PLD. Additionally, in AE $G_{q/11}$ antibody inhibition of ACh contraction was not augmented by M_3 and PI-PLC antagonists, and the inhibition by G_{i3} antibody was not augmented by M_2 and PC-PLC or PLD antagonist.

We conclude that in normal LES cells contraction is mediated by M_3 receptors linked to $G_{q/11}$ and PI-PLC, while in esophagitis, contraction through this pathway is reduced since IP_3 production and Ca^{++} stores are damaged. A second pathway mediated by M_2 receptors linked to G_{i3}, PC-PLC and PLD is activated after esophagitis (Sohn et al., 1994).

Cellular Ca^{++} measurement and receptor binding study are required for observing detailed mechanism. We are developing chronic esophagitis model induced by myotomy.

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