PHARMACOLOGY OF THE ESOPHAGEAL MOTOR FUNCTION

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OPERATION AND STRUCTURE OF THE ESOPHAGUS

The esophagus comprises three functionally distinct regions: the upper and lower esophageal sphincters and the intervening esophageal body. At rest, the upper esophageal sphincter (the most proximal 10% of the organ) and the lower sphincter (the most distal 10%) are both tonically contracted to produce luminal occlusion, while the esophageal body is flaccid. In swallowing, both sphincters relax. The upper sphincter closes after about a second of relaxation; its closure is followed by a progressive contraction along the esophageal body, esophageal peristalsis. The arrival of this peristaltic contraction at the lower sphincter produces its closure. For a detailed account of the physiology and anatomy of the esophagus see the review by Ingelfinger (1).

In humans, the proximal third of both layers of the muscularis propria is striated muscle, the rest is smooth muscle. These distributions differ among species (2). Some marsupials and primates share the human proportions, while the esophagus is wholly smooth muscle in amphibia, birds, and reptiles. Only the most distal fifth or less is smooth muscle in common laboratory animals. The muscularis mucosae is smooth muscle in all species.

The esophagus receives both a cranial and a thoracic innervation. The nerves are distributed through a myenteric plexus which is present even in the striated muscle segments except probably for the upper esophageal sphincter. The myenteric plexus contains both argyrophilic and argyrophobic ganglion cells (3), and catecholamine-fluorescent fibers (4, 5) which end in an apparently synaptic relationship with ganglion cells. There is also some direct adrenergic innervation of both layers of the muscularis propria, even in striated muscle (at least in the rabbit). Adrenergic fibers also end in the muscularis mucosae and in the sparse submucosal plexus, but they are absent in the circular muscle layer of the lower esophageal sphincter (of monkey). The argyrophilic ganglion cells, considered to be cholinergic (3), are lost in achalasia, a disorder generally attributed to denervation of the esophagus.

Evidence reviewed below indicates that the esophagus has both an excitatory and an inhibitory innervation. Although both cholinergic and adrenergic nerves are present, pharmacologic evidence suggests that other kinds of nerves may be important in the regulation of esophageal movement.

THE PHARMACOLOGY OF ESOPHAGEAL STRIATED MUSCLE

The motor nerves to striated muscle are thought to arise in the dorsal motor nucleus and nucleus ambiguus and to pass through the vagi without synaptic interruption, ending in motor endplates like those of somatic muscle (1, 6). The tonic closure of the upper sphincter is accompanied by constant discharge of muscle action potentials (7) which disappear transiently with swallowing. Apparently, the upper sphincter is contracted by tonic discharge of these nerves and relaxation represents transient central inhibition, for transient inhibition precedes contraction of the somatic neck muscles activated early in swallowing (8). In the striated muscle of the esophageal body, the somatic nerves are assumed to be activated in coordination with, and just after, inhibition of the somatic nerves to the upper sphincter. This view of the innervation may not be complete, for the striated muscle does not atrophy for up to 9 months after cervical vagal section and still can contract (9). The explanation for this curious observation is not apparent.

The study of the effects of drugs on the upper sphincter muscle has been neglected.

In the striated muscle of the esophageal body in dogs, contractions induced by vagal stimulation are inhibited by curare but not by atropine and nicotine (10), nor are swallowing-induced contractions in this region in humans weakened by atropine (11). Acetylcholine-induced contractions of strips of striated muscle of guinea pigs are suppressed by tubocurarine. Methacholine is a poor agonist in such preparations (12). In the guinea pig, contractions of striated muscle induced by vagal stimulation also are sensitive to tubocurarine and decamethonium but insensitive to hexamethonium and hyoscine. Decamethonium itself excites contractions (13). Prolonged administration of diisopropylfluorophosphate to the dog induces paralysis of the striated muscle (14), and dogs so treated develop dysphagia (14, 15).

This sparse evidence all supports the view that neuromuscular transmission in this muscle is cholinergic, and nicotinic rather than muscarinic.

THE PHARMACOLOGY OF SMOOTH MUSCLE OF THE ESOPHAGEAL BODY

Cholinergic Antagonists

Anticholinergic agents are thought generally to inhibit contractions in the smooth muscle. Atropine, in total doses of 0.65 and 2.0 mg, reduces the force of swallowing-induced contractions of the esophageal body registered by esophageal manometry in humans, and reduces the proportion of swallows that induce contractions (11). Even though large doses of atropine may abolish contractions as registered manometrically, contractions still may be seen radiographically (16). This illustrates one problem with manometry: the range of detection of contractions by measurement

of intraluminal pressures may not encompass the full range of contraction forces. Other studies report that atropine does not consistently affect esophageal peristalsis in humans (17, 18). Anticholinergics do seem to reduce the amplitude of contractions excited by balloon-distension of the distal esophagus in humans (19–21). Thus, the evidence is inconsistent as to the effect of anticholinergics on contractions of esophageal smooth muscle as studied in vivo. This probably represents the inadequacy of the methods. It seems reasonable to conclude that such agents may appreciably weaken contractions but, in the doses used, do not abolish them.

The long smooth-muscled segment of the esophageal body of the opossum can be made to contract in vitro, either reflexly by distension or by electrical excitation (22, 23). With both kinds of stimulation, a peristaltic contraction sweeps the length of the esophagus after the stimulus is terminated. This is abolished by tetrodotoxin in low concentrations and appears to involve only the inner circular layer of the muscularis propria. These contractions in vitro are insensitive to atropine (23).

Isolated strips of smooth muscle from the esophageal body of the opossum, subjected to electrical field stimulation, respond with tetrodotoxin-sensitive contractions. In such strips, the three muscle layers respond differently. The longitudinal muscle of the muscularis propria and the muscularis mucosae both contract with the onset of a train of short pulses and remain contracted until the end of the train. These contractions are atropine-sensitive. The circular layer of the muscularis propria contracts briefly only after the end of the train (with a delay of 0.9–2.2 sec) and this contraction is not affected by atropine (24). This twitch, called the off-response, is the only kind of nerve-mediated (or at least tetrodotoxin-sensitive) contraction response that can be produced by electrical field stimulation in the circular muscle layer of the esophageal body. The same kind of response also occurs in similar preparations of the cat and rhesus esophagus (25). In the isolated chick esophagus, contractions of the longitudinal layer of the muscularis propria induced by stimulation of the vagus are sensitive to atropine (26), and hyoscine-resistant contractions of the circular muscle layer in response to vagal stimulation have been reported in chickens (27, 28).

The evidence reviewed above, though confined to a few species, is consistent with the hypothesis that the smooth muscle of the esophageal body receives at least two kinds of motor innervation. One, that to the longitudinal muscle layer and to the muscularis mucosae, is cholinergic and muscarinic, being atropine-sensitive. The other, that to the circular muscle layer, is atropine-resistant (and resistant as well to adrenergic antagonists). Its transmitter is obscure in nature. As it is conceivable that contractions of all three layers may be detected by manometry and fluoroscopy in vivo, and as all layers may be activated in swallowing, this view may explain the inconsistencies of the results of studies with anticholinergic agents in vivo in humans.

Cholinergic Agonists

These agents seem to have a minor effect on smooth muscle of the human esophageal body in vivo. Bethanechol slightly augments balloon-induced contractions (20) but does not affect esophageal transit time (29). Methacholine modestly raises resting

intraluminal pressure in the human distal esophagus (30). Balloon-induced contractions are little affected by methacholine. Prostigmine and physostigmine have little effect in normal humans (17, 31). In patients with achalasia, however, both methacholine and bethanechol induce vigorous contractions of the smooth muscle (20, 29–33). This effect is sought commonly to diagnose achalasia, and the response is attributed to postdenervation hypersensitivity (32). Loss of ganglion cells in the myenteric plexus has been demonstrated anatomically in both idiopathic achalasia and that variety associated with Chagas' disease (1, 3).

In isolated strips of body smooth muscle from the esophageal body of cat and opossum, acetylcholine, methacholine, and carbachol induce contractions in both layers of the muscularis propria, and these contractions are atropine-sensitive (34–38). The longitudinal and circular muscle layers in the cat appear to be about equally sensitive to acetylcholine (34, 35). Both layers of the muscularis propria and the muscularis mucosae of the fetal human esophagus are contracted in vitro by acetylcholine (38).

Adrenergic Agonists and Antagonists

Adrenergic agents in general have little effect upon normal human esophageal body function in vivo (18, 31). Epinephrine, given parenterally to normal subjects in doses sufficient to produce moderate cardiovascular effects, has no appreciable effect on contractions of the esophageal body or upon esophageal transit. Dibenzyline and phentolamine also have no effect on contractions of the distal esophageal body (31).

Isolated strips of muscle from the esophageal body of cats, cut so as to reflect activity of both the longitudinal and circular muscle layers separately, are contracted by epinephrine and norepinephrine, and these contractions are antagonized by tolazoline and by other α antagonists. Isopropylnorepinephrine inhibits both layers, and this inhibition is opposed by propranolol (34, 35). The conclusions that adrenergic α receptors are excitatory and β receptors are inhibitory throughout the esophageal body is supported by evidence from studies of muscle in vitro from the opossum (37) and guinea pig (39). But when strips of muscle from the esophageal body of the opossum are subjected to electrical field stimulation, none of the tetrodotoxin-sensitive responses of any of the three layers are opposed by antagonists of transmission at either adrenergic α or β receptors (24), probably because the adrenergic nerves known to be present cannot be excited.

These results imply that the adrenergic nerves, known to be present in the esophageal body from histochemical studies (4, 5), may be either excitatory or inhibitory in nature, depending upon which kind of adrenergic receptor they reach (40). The catecholamine-fluorescent fibers seem to end on both neural and muscular structures. The relative distributions of the two adrenergic receptor types among nerves and muscles has not been determined, although there are suggestions that at least some excitatory α receptors may be located in cholinergic neurons (34). The available evidence, though sparse, does not suggest that the adrenergic nerves are principally responsible for esophageal body motor function, and their exact role remains to be defined.

Agents Affecting Ganglionic Transmission

The ganglionic antagonist, trimethaphan, given to humans in a dosage sufficient to cause systemic effects, inhibits propulsion of a distended balloon along the esophagus (21). In the isolated whole esophagus preparation of the opossum, hexamethonium has no effect on the peristaltic contraction induced by electrical excitation of the intramural nerves or upon that reflexly produced by balloon distension of the esophagus (23). In transverse strips of muscle cut from the esophageal body of the opossum, the off-response to electrical field stimulation is not abolished by hexamethonium (24, 41). These results suggest that the peristaltic response to swallowing in the smooth muscle segment of the esophageal body does not involve a ganglionic transmission process sensitive to hexamethonium.

Both nicotine and dimethylphenylpiperazinium excite contractions in vitro in the circular smooth muscle of the opossum esophagus, and the effect is dose-related (37). The site of action of these agents is not known.

Other Agents

In humans, metoclopramide increases the force and duration of swallowing-induced peristaltic contractions in the smooth-muscled segment of the esophageal body, as they are recorded manometrically (42). Gastrin, given subcutaneously in large doses to humans, does not affect the force or duration of esophageal peristaltic contractions (43). In the isolated innervated chick esophagus, contractions in the long axis (apparently of the longitudinal muscle layer) induced by coaxial electrical stimulation and by stimulation of the attached parasympathetic nerves, are depressed by a variety of narcotic analgesics (morphine, diacetylmorphine, etorphine, methadone, meperidine, propoxyphene, pentazocine, cyclazocine, and phenazocine). The responses of this preparation to acetylcholine and to 5-hydroxytryptamine are not opposed by the agents, except for meperidine, propoxyphene, and pentazocine, so the depression appears probably to be exerted prejunctionally (44). In the human esophagus, intravenous morphine causes a relaxation of tone in the distal esophageal body, as recorded by balloon kymography (18).

Amyl nitrite and nitroglycerine inhibit contractions of the distal esophagus in normal humans (19) and in patients with diffuse spasm of the esophagus (45).

THE PHARMACOLOGY OF SMOOTH MUSCLE OF THE LOWER ESOPHAGEAL SPHINCTER

The Problem of Identifying the Lower Esophageal Sphincter

The existence of an esophagogastric sphincter, a mechanism to separate the esophageal and gastric lumens, implies the existence of control systems to this junction which differ in some respects from those for the muscle of the esophageal body and proximal stomach. The study of these control systems requires methods to examine individually the two distinctive functions of the sphincter: tonic closure at rest and relaxation upon swallowing.

A variety of methods has been used to examine these functions. Fluoroscopic observation of the luminal diameter and of flow through the junction are purely qualitative. In whole animals, balloons can be inserted into the region and inflated, and pressures in the balloons are assumed to reflect the tension in the sphincteric segment. Balloon kymography presents at least three problems. First, the balloon, if large, may be affected by regions adjacent to the sphincter, as well as by the sphincter itself. Second, the balloon, if small, may not maintain a constant position relative to the sphincter. Third, the distension of the balloon may alter the measurements made.

In esophageal manometry, pressure is recorded from a small flexible tube with a distal lateral opening while the tube is perfused at a constant rate with water. The pressure recorded is assumed to be that required to force the sphincteric wall away from the distal opening so that flow can occur, and this pressure is assumed to reflect the force of contraction in the wall of the sphincteric region. Two techniques are used. In one, the catheter is positioned so that the distal opening remains in the sphincteric segment over long periods of time. In this case, the assumption is made that catheter shifts do not occur, an assumption which may or may not be true. In the second method, the catheter is pulled slowly from the stomach into the esophageal body to produce a "pressure profile" across the sphincter. Such sampling allows only a very rough appreciation of time-dependent variations in the force of closure of the sphincter, and it appears that time-dependent variations are probably quite large in humans.

Recently, the sphincter has been examined by studying isolated muscle strips. With such techniques it is critical to distinguish sphincter from nonsphincter with a high degree of resolution. Such methods also have the familiar problem that the activity of the sphincteric muscle may be altered by its isolation.

Cholinergic Antagonists

Resting lower esophageal sphincter closure tension, recorded manometrically in humans, is reduced by atropine, 0.025 mg/kg given parenterally, but not abolished (46). A dose of 1.2 mg of atropine, given parenterally, can reduce resting closure tension sufficiently to increase gastroesophageal reflux (47). Propantheline also reduces resting closure tension in the human esophagogastric sphincter (48). The subject is not without controversy, however, for a double-blind study of another anticholinergic agent, valethamate bromide, failed to show any effect of the prolonged oral administration of that agent upon resting closure tension in normal subjects studied manometrically (49). It is surprising that so few investigations have been made of the effects of anticholinergic agents upon sphincteric closure tension in man. The evidence suggests only that anticholinergics, if they have any effect at all, may reduce but do not abolish closure at the sphincteric region.

The uncertainty about the ability of anticholinergic agents to antagonize sphincteric closure tension is supported by studies of the region in situ in animals. In the perfused esophagus-stomach of the cat, hyoscine and atropine given intravenously have little effect on the tone of the sphincter (50). On the other hand, in the intact

dog, subcutaneous atropine nearly abolishes sphincter closure tension as recorded manometrically (51).

The sphincter region can be made to increase the force of its tonic contraction in situ by abdominal compression, and this response is opposed by atropine in humans (46). On deep inspiration, sphincter closure tension, recorded manometrically in humans, falls below the tension recorded in the normal respiratory cycle (the tension varies over a narrow range with quiet respiration, falling in inspiration) and the level to which it falls is not affected by parenteral propantheline (48). In the decerebrate, pithed, or anesthetized cat, contractions of the distal esophagus (presumably the sphincter region) are produced by distension, by perfusion of the lumen with HCl, and by stimulation of the central ends of the cut vagi. These contractions are abolished by parenteral atropine (52). These observations support the view that there are cholinergic fibers innervating the sphincter that are capable of increasing sphincter closure tension. This conclusion, however, suffers the restriction that such studies in whole animals may not clearly distinguish sphincter from nonsphincter.

Isolated transverse muscle strips, cut from the region of the esophagogastric junction in the cat, opossum, and rhesus monkey, and subjected to electrical field stimulation with bursts of short pulses at low frequencies, respond with contraction and relaxation that are tetrodotoxin-sensitive and hence probably neural (25, 53). When serial strips 2 mm wide are cut from a level well above to a level well below the junctional segment, comparison of their responses to such excitation of intramural nerves reveals a distinctive innervation of the immediate junctional region. Strips from the esophageal body neither contract nor relax during the pulse train, but contract briefly about 2 sec after the end of the stimulus train. This is called the off-response. Strips from the proximal stomach contract at the onset of the train and remain contracted until the end. But in the junctional region, strips never contract but only relax during the train. There are transitional strips both at the top and bottom of this purely relaxing zone in which relaxation is followed by an off-response (at the proximal end of the relaxing zone) and in which relaxation and contraction both occur during the pulse train (at the distal limit of the relaxing region). The region showing only relaxation is less than 1 cm long in the cat and opossum, a little longer in the rhesus. The contractions occurring during the stimulus train in the strips just below the relaxing zone are cholinergic, for they are antagonized by atropine and potentiated by physostigmine (J. Christensen, unpublished). It seems possible that manometry and balloon kymography of the sphincter in situ may not be capable of distinguishing resting closure tension in the purely relaxing zone from that in the contracting region immediately below it, accounting for the conflicting observations as to the ability of anticholinergic agents to alter resting closure tension at the junction. That is to say, the sphincter as observed manometrically or kymographically may include a distal zone with a cholinergic motor innervation and a proximal one without it.

A decrease in sphincter closure tension is associated with swallowing and can be induced by vagal stimulation in various animals. In the sphincter in situ in the cat, the relaxation induced by vagal stimulation is abolished by large doses of hyoscine

and atropine (50). Sphincteric relaxation in situ in the rabbit, induced by vagal stimulation, is opposed by atropine (54), and methantheline seems to antagonize this effect (55). In isolated muscle strips cut from the junctional regions, the relaxation induced by electrical field stimulation is neither antagonized by atropine nor potentiated by physostigmine (41; J. Christensen, unpublished observations). Conceivably, the apparent antagonism to swallowing relaxation in animals in vivo produced by atropine and similar agents may simply result from a reduction in the level of tonic contraction by the anticholinergic agents and a consequent reduction in the ability of the sphincter to express the inhibition. It could be, as well, that the impairment of sphincteric relaxation produced in vivo by cholinergic antagonists reflects an action of those agents on ganglionic transmission or in the central nervous system. Certainly, in clinical therapy, atropine and other anticholinergic agents do not alter sphincteric relaxation sufficiently with swallowing to affect patients' perception of swallowing or to alter the esophagram.

Cholinergic Agonists

In humans, bethanechol given parenterally raises the baseline closure tension of the lower esophageal sphincter as recorded manometrically (47, 56), at least in some (but not all) subjects. As parenterally administered cholinergics may induce many effects, the mechanism of this effect is not clear. In the esophagus of the cat in vivo, acetylcholine given parenterally causes sphincteric relaxation, and this effect is potentiated by physostigmine, antagonized by atropine, and unaffected by hexamethonium (50). Transverse muscle strips from the sphincter of the cat are inhibited by acetylcholine according to one study (57), but excited according to another (36). Similar strips cut from the opossum esophagus are excited to contract by acetylcholine, methacholine, and carbachol (37). Longitudinal muscle strips cut from the region of the sphincter in humans are contracted by acetylcholine (58). As they stand, these observations do not permit a conclusion that muscarinic receptors are inhibitory in the sphincter. In the experiments in vivo, the recording methods probably cannot reliably distinguish sphincter from nonsphincter, if one defines the sphincter as the zone of pure relaxation with electrical field stimulation (25). What is needed is an examination of the effect of cholinergic agonists on the muscle strips cut exclusively from the region that relaxes in response to electrical field stimulation. This simple study has not been done because that region has only very recently been described (53).

Adrenergic Antagonists and Agonists

Although patients frequently receive adrenergic antagonists, symptoms of esophagogastric dysfunction are rare in such cases. Experimental studies of the effects of adrenergic agents on sphincteric function in humans are limited by the cardiovascular effects of adrenergic agents. In humans, sphincter closure tension as recorded manometrically is not affected by phentolamine. Alprenolol seems to have little effect on resting closure tension but does induce an increase in the force and duration of sphincteric contraction that follows relaxation with swallowing (59). In the opossum sphincter, observed manometrically, phentolamine reduces resting closure

tension but propranolol has no effect, and 6-hydroxydopamine reduces resting closure tension as well (60). Dibenamine can reduce the closure tension of the sphincter in achalasia (61).

Phenylephrine has no effect on resting closure tension in the sphincter in humans, but isoproterenol reduces it, according to one manometric study (59). Both norepinephrine and phenylephrine raise closure tension and isopropylnorephrine reduces it, according to others (60). These few studies suggest that closure tension may be raised through stimulation of excitatory α receptors and reduced by inhibitory β receptor stimulation.

These inferences are supported by studies of other kinds of preparations. In isolated muscle strips cut transversely from the opossum esophagus, norepinephrine excites contractions in the most distal 1 cm of the esophagus (just as it does at higher levels), and this response is enhanced by propranolol (37). In human esophagus, transverse muscle strips cut from the probable sphincteric region are both contracted and relaxed by norepinephrine but adrenergic α antagonists convert this response to inhibition alone. Isopropylnorepinephrine in such strips reduces tension and this effect is opposed by pronethalol (62). In the human esophagus, transverse strips of muscle from the sphincter are contracted by norepinephrine, but relaxed by norepinephrine in the presence of phentolamine (63). In the perfused cat esophagus in situ, both epinephrine and norepinephrine given intravenously cause contraction of the sphincteric segment, and isopropylnorepinephrine produces relaxation of the region (50). In isolated transverse strips of muscle cut from the sphincteric region of the cat, norepinephrine induces contractions (36), as does epinephrine (47). But in the rabbit sphincter, both adrenergic α agonists and β agonists induce relaxation (64).

Thus, these observations quite consistently support the conclusion that the sphincter, like the esophageal body in most species including humans, possesses excitatory adrenergic α receptors and inhibitory adrenergic β receptors. Adrenergic nerves appear to be sparse in the region, at least in some species (4, 5). Adrenergic mechanisms may be physiologically significant in the normal control of resting sphincteric closure tension, although that has not been demonstrated, but they are probably not the only functionally significant control system.

Neurogenic relaxation of the sphincteric muscle of the opossum and cat, induced by electrical field stimulation, is apparently not adrenergic. At least, this relaxation is not opposed by a variety of α antagonists nor by propranolol (41; J. Christensen, unpublished). In manometric studies of the opossum, the sphincteric relaxation produced by swallowing is not opposed by propranolol, phentolamine, or 6-hydroxydopamine (60). Thus, neurogenic inhibition produced by swallowing probably is not adrenergic.

The Gastrointestinal Hormones

GASTRIN Intravenous bolus injections of gastrin raise basal closure tension in the lower esophageal sphincter in humans. In the first report of this effect (65) unperfused manometric catheters were used, a technique that yields lower values for pressure (reflecting closure tension) than perfused catheters. The catheters were

pulled slowly through the sphincteric region before and 15 min after graded bolus injections. The data, presented without statistical analysis, suggest the effect but a dose-effect relationship was not demonstrated. Atropine prevented the response. Gastrin pentapeptide, constantly infused intravenously, was inactive. The introduction of meat extract into the stomach also seemed to raise sphincteric closure tension. A subsequent study in humans (66) by perfusion manometry reported that subcutaneous injection of pentagastrin, 1 μg/kg, raises pressures recorded from the sphincter with a peak change at 7 min. In that same study, the intragastric instillation of alkali, peptone broth, or 10% ethanol, and the ingestion of a ground beef meal (all procedures which, among other things, may cause endogenous gastrin release) raised closure tension in the sphincteric region. Intragastric instillation of HCl caused a fall in closure tension. Although these observations can be interpreted as being consistent with the idea that both exogenous and endogenous gastrin increase sphincteric closure tension, the lack of saline controls and the absence of rigorous statistical treatment limit the certainty of the conclusion.

Subsequently, synthetic gastrin I (residues 2 to 17) was given to normal subjects by bolus intravenous injection during infusion manometry with the tube fixed at the lower esophageal sphincter (67). Pressures at the peak of the rise after injection were expressed in terms of percent of basal pressures, and injections were made in graded doses from 0.025 to 1.5 μg/kg. The resulting dose-effect curve was roughly sigmoid on a semilog plot with the peak, a nearly fivefold increase in pressure, occurring at 0.7 μg/kg. Appropriate statistics indicated that the effect was significant. Saline controls were not reported.

The ability of exogenous pentagastrin to alter sphincteric closure tension is not questioned, but the dosage and route of administration are critical. One-hour continuous infusions of pentagastrin at 0.015 and 0.025 μg/kg per min were made in humans and sphincteric closure tension was recorded by pulling infused manometric catheters through the sphincteric region at 10 min intervals while gastric acid was continuously aspirated (68). Bolus injections of 0.5 μg/kg were also made. Minimal rises were seen with constant infusion at doses which maximally stimulate gastric secretion, but the bolus injections produced twofold peak changes. That study (in abstract only) did not report saline controls.

The claim of the ability of endogenous gastrin to affect sphincteric closure tension is challenged. In a recent study (in abstract) sphincter closure tension was recorded continuously with infused tubes anchored in the sphincter in normal humans while 0.1 N HCl and $NaHCO_3$ were instilled into the stomach in random double-blind fashion (69). Intragastric pH was measured continuously. Sphincteric pressures were read blindly. No significant differences in closure tension were detected between the conditions of acid and alkali infusions over a 30 min period. Yet another study (70) reports that intragastric instillation of 0.1 N HCl in normal subjects lowers resting closure tension as recorded by infusion manometry, but statistical evidence of significance was not presented.

The subject is confused further by another study in humans that suggested that local chemoreceptors may reflexly alter sphincteric closure. Nonperfusion manometry was done by pulling a tube through the sphincter segment while the mucosal

surface was continuously perfused by a second tube whose hole was just above that of the pressure-sensing tube (70). Presumably, the perfused fluids escaped into the stomach. The fluids included saline, 0.05 N HCl, and buffered solutions of pH 1.0 to 8.0. The conclusion was reached that exposure of the mucosa in the sphincter to an acid pH raises closure tension, with a peak effect at pH 3.0. Atropine prevented the effect. Possible suppression of endogenous gastrin release and release of secretin were not considered, though the solutions certainly must have reached the gastric and duodenal lumen. Cannon long ago had proposed that acid in the lumen of the sphincter is important in controlling closure tension. This suggestion was further addressed by a study in the perfused esophagus preparation in the cat (50). The esophagus was studied in vivo in a preparation in which only the distal esophagus and proximal stomach were perfused. The antrum was not exposed to perfusing fluids. When various concentrations of HCl, 0.01–0.1 N, were allowed to flow through the sphincter, closure tension increased in proportion to concentration, and this effect was not changed by bilateral vagotomy. Alkaline fluids reduced closure tension. Injections of 0.1 N HCl into the pouch of the proximal stomach also raised sphincter closure tension. These results suggest a local mechanism, neural or hormonal, by which mucosal chemoreceptors affect sphincter closure, tension rising with increasing concentrations of hydrogen ion. The effect was attributed tentatively to local reflexes arising from mucosal receptors at or just below the sphincter.

The ability of endogenous gastrin to affect sphincteric closure tension was also tested immunologically (71). Antibodies to synthetic human gastrin I (residues 2 to 17) were prepared in rabbits. A control antiserum to thyroid-stimulating hormone was similarly prepared. Infusion manometry was used to study basal closure tension in anesthetized opossums. Intravenous pulse injections of antiserum lowered resting tension and the effect was dose-related, the maximum reduction occurring with 2.0 ml of antiserum, while the control antiserum had no effect. Sphincter closure tension was raised by increasing gastric pH to 7, and the gastrin antiserum, but not the TSH antiserum, antagonized this rise significantly. When closure tension was raised by intravenous bolus injections of gastrin I, the gastrin antiserum, but not the TSH antiserum, significantly reduced the response. Although gastrin antiserum saturated with gastrin might have been a more suitable control, this study seems to provide good evidence that gastrin, in the opossum, is important in the maintenance of sphincteric closure tension at rest.

There is little doubt that parenteral bolus injections of gastrin raise closure tension. Whether or not endogenous gastrin has an important physiological role in maintenance of closure tension is still in question. Because serum gastrin concentrations after bolus injections are transiently far higher than those that occur with normal physiological stimulation, Grossman believes that the response to gastrin delivered as an intravenous bolus is a pharmacological, rather than a physiological effect (71).

Pathologically high serum levels of gastrin occur in patients with certain diseases, and if gastrin is physiologically significant in the control of closure tension, such patients might be found to have elevated tensions. In one study, patients with the Zollinger-Ellison Syndrome (a disorder characterized by very high serum levels of

gastrin) had higher resting closure tension in the sphincter than normals (72), but in another study they did not (73). Intravenous bolus injections of gastrin raise sphincteric closure tension in such patients but apparently less than in normal subjects.

Gastrin causes contraction of the muscle of the sphincteric region in vitro (74). In isolated muscle strips cut from the opossum esophagus, synthetic human gastrin I induces a dose-related contraction with a lower threshold concentration and a higher peak active tension in muscle strips cut from the sphincteric region than it does in strips cut from the midesophagus, the gastric fundus, and antrum. Gastrin I seems to be very potent, threshold concentrations being about 10^{-13} *M*. The contractions caused by gastrin I in sphincteric muscle in vitro are antagonized by atropine, hyoscine, tetrodotoxin, and hemicholinium. Hexamethonium, pentolinium, propranolol, phentolamine, methysergide, and diphenhydramine have no effect. The gastrin effect is potentiated by physostigmine. These results suggest that gastrin excites acetylcholine release at a cholinergic neuroeffector (75). This cholinergic neuroeffector remains hypothetical, for it has not yet been demonstrated to exist by techniques of nerve stimulation.

Thus, gastrin excites the muscle of the sphincter, as it does other gastrointestinal muscle. The view that the endogenous release of gastrin is a major factor in moment-to-moment control of sphincter closure tension has not achieved general acceptance and remains a matter of controversy (71, 76). Recently, it has been proposed that the abnormal sphincteric closure tension in patients with various disorders (reflux esophagitis, achalasia) reflects abnormal release or abnormal sensitivity to endogenous gastrin. The validity of these hypotheses rests on resolution of the controversy about whether the normal sphincter responds to endogenously released gastrin.

SECRETIN Acidification of the duodenum, which releases endogenous secretin, does not significantly change sphincteric closure tension, as determined by infusion manometry in humans (67, 79). Neither do bolus injections and constant intravenous infusions of secretin (67, 79). Both procedures, however, reduce the response to bolus injection of gastrin I. Also, intravenous bolus injections of secretin, 0.1–0.5 units/kg, produce a transient fall in sphincter closure tension that is dose-related under conditions of gastric alkalinization. These observations have led to the conclusion that secretin competitively inhibits gastrin excitation of sphincteric closure tension (67). In isolated strips of muscle cut transversely from the region of the sphincter in the opossum and exposed to secretin in vitro, secretin has an insignificant effect on resting tension, but it does antagonize responses to gastrin in vitro (77). Secretin may compete with gastrin at the common receptor postulated to exist for gastrin, secretin, and cholecystokinin (77, 78).

In view of the lack of evidence that endogenously released secretin significantly affects resting sphincter closure tension in humans, and that secretion has an effect on resting tension in transverse muscle strips from the sphincter region in the opossum, it seems unlikely that secretion is important physiologically in regulation of sphincteric closure tension. It acts as a gastrin antagonist, and so any conclusion about its physiological importance rests on the validity of the conclusion that endogenous gastrin alters sphincteric closure tension.

CHOLECYSTOKININ Endogenous cholecystokinin is released by a fatty meal. In normal subjects the ingestion of a fatty meal causes a significant fall in sphincter closure tension as monitored by anchored manometric tubes, and the introduodenal instillation of corn oil has the same effect (79). When normal subjects are given gastrin I in bolus intravenous injections while the resting closure tension in the sphincter is monitored, the ingestion of a fatty meal shifts the dose-effect curve to the right in parallel (80). The synthetic C-terminal octapeptide of cholecystokinin, in bolus intravenous injections, produces a dose-related reduction in lower esophageal sphincter pressure as measured by infused manometric tubes fixed in sphincter (81), while control injections have no effect. The action begins within 30 min. In both humans and the opossum, bolus injections of cholecystokinin reduce manometrically recorded resting closure tension in the sphincter. In contrast, cholecystokinin and the octapeptide of cholecystokinin are partial agonists in sphincteric muscle in vitro, but they antagonize the excitation produced by gastrin in vitro (82). Cholecystokinin may function as a competitive inhibitor of gastrin (82), both agents acting at the proposed common receptor (78).

Fat in the duodenum could relax the sphincter by either neural or hormonal mechanisms. An enterogastrone, released from the duodenum by fat, could be important physiologically in the regulation of sphincteric closure. Whether or not it is cholecystokinin remains to be established. Any conclusion about the physiological significance of this putative enterogastrone in regulation of tonic closure also rests on the validity of the conclusion that endogenous gastrin has such a role.

Agents Affecting Ganglionic Transmission

In the perfused esophagus preparation of the cat studied in vivo, intravenous bolus injections of nicotine induces either relaxation or contraction of the sphincter, and hexamethonium reduces resting closure tension (50). In isolated transverse strips of muscle cut from the opossum esophagus, nicotine and dimethylphenylpiperazinium induce contractions in the lowest centimeter of the esophagus as well as at all levels of the esophagus, but the sensitivity of the lowest centimeter is greater (37). In isolated strips of muscle from the sphincter region in humans, both nicotine and dimethylphenylpiperazinium produce relaxation which is antagonized by hexamethonium, bretylium, pronetholol, and phentolamine (62). In another study using isolated muscle strips in vitro (63), the circular muscle layer of human sphincter was relaxed by nicotine, but muscle above the cardia was contracted. Isolated strips of the longitudinal muscle layer from this region in humans are not excited by nicotine (58).

Other Agents

In the sphincter of the anesthetized opossum, studied by infusion manometry, the intravenous bolus injection of prostaglandin F_2 usually raised closure tension, but in some cases there was a fall in tension (83). In similar experiments in opossums (84), prostaglandin E_1, given as an intravenous bolus or by close intraarterial injection, produced a dose-related fall in sphincter closure tension that was not affected by anticholinergic agents, adrenergic antagonists, or gastrin. Relaxation was also produced by isoproterenol, theophylline, and dibutyryl cyclic AMP, and theophyl-

line and dibutyryl cyclic AMP potentiated the effect of prostaglandin E_1. Nicotinic acid, an adenylcyclase inhibitor, and imidazole, a phosphodiesterase stimulator, inhibited the relaxation produced by prostaglandin E_1 (84).

Metoclopramide, an agent whose mode of action is not known, increases closure tension in the sphincter as monitored manometrically in humans (42). The oral administration of metoclopramide similarly raises closure tension slightly but significantly (85).

The intragastric instillation of caffeine sodium benzoate, which stimulates gastric acid secretion, produces, if anything, a fall in sphincter closure tension. Relaxation of sphincter closure tension is also reported to be produced by intragastric instillation of oil of peppermint (86).

SUMMARY

In the striated muscle of the upper esophageal sphincter, tonic maintenance of closure is probably mediated via tonic central excitation of the extrinsic motor innervation; relaxation represents central inhibition of this mechanism. The motor nerves are probably cholinergic and act through nicotinic receptors like those of somatic striated muscle.

In the striated muscle of the esophageal body, swallowing-induced contraction is also probably a cholinergic and nicotinic response.

In the smooth muscle of the esophageal body, the control of contractions is cholinergic and muscarinic in part, but there is evidence for a nonadrenergic and noncholinergic component as well. The muscarinic component may arise from the cholinergic innervation of the longitudinal muscle layer. The other component may lie in the cryptic innervation of the circular muscular layer.

In the smooth-muscled lower esophageal sphincter, resting closure tension appears to reflect a variety of possible control mechanisms. No single control system predominates. The evidence for muscarinic excitation is equivocal. An excitatory adrenergic α mechanism and inhibitory adrenergic β receptors may contribute. A role for the polypeptide hormones from the gastrointestinal tract seems unlikely. Relaxation of the lower sphincter with swallowing seems not to involve any of these mechanisms, but is apparently accomplished by nonadrenergic noncholinergic inhibitory nerves like those present elsewhere in the gut (87). The possibility that the transmitter of these nerves is an adenine nucleotide has been raised from studies of other parts of the gut, but that hypothesis has not yet been examined critically in the lower esophageal sphincter.

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