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Research Article

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ABSTRACT To determine the pathogenesis of esophageal dysfunction in scleroderma and Raynaud's disease, the lower esophageal sphincter (LES) was tested with: (a) methacholine acting directly at the cholinergic receptor on the muscle; (b) edrophonium, a cholinesterase inhibitor, enhancing the effect of released acetylcholine; and (c) gastrin I, acting through the release of acetylcholine. 10 patients with Raynaud's disease and 22 patients with scleroderma were compared with 20 normals and 20 patients with isolated LES incompetence. The mean basal LES pressure in normals was significantly greater than that recorded in both patients with scleroderma and Raynaud's disease. Six patients having scleroderma with normal peristalsis had an LES pressure significantly greater than that noted in 16 patients having scleroderma with abnormal peristalsis. In all groups, the per cent increase in LES pressure was similar when tested by direct muscle stimulation by methacholine. The response to agents that acted indirectly through intact cholinergic nerves differed in these groups. The LES response to gastrin I distinguished patients with normal peristalsis from those with abnormal peristalsis. The patients with normal peristalsis, either with scleroderma or with Raynaud's disease showed only a partial reduction in their response to gastrin I. The response to gastrin I was markedly reduced only in patients with abnormal peristalsis. These data indicate that in patients with scleroderma and Raynaud's disease, the LES response to direct muscle stimulation by methacholine was intact while the response to gastrin I and edrophonium was diminished.

INTRODUCTION

In patients with scleroderma and Raynaud's disease, the esophagus shows functional abnormalities in its smooth

muscle portions (1-6). Peristaltic waves diminish in amplitude and then disappear in the distal one-half to two-thirds of the esophagus. The physiologic lower esophageal sphincter (LES)¹ becomes incompetent. At postmortem examination, the smooth muscle is atrophied but collagen deposition and fibrosis are not prominent (1, 7). The pathogenesis of these smooth muscle changes is not known. It has been suggested that either the muscle atrophy may be primary or that atrophy may be secondary to a neurogenic cause (1, 2). Several observations support the neurogenic hypothesis. First, functional derangements precede pathological changes as noted by comparison of manometric esophageal recordings and postmortem examination for muscle atrophy by light microscopy (1). Second, esophageal dysfunction correlates closely with Raynaud's phenomenon suggesting that a common neurogenic abnormality may cause both disorders (2). Third, in a small group of patients, abnormalities in esophageal function have been reversed by intra-arterial reserpine suggesting that muscle function is preserved (4). The purpose of this study is to utilize a series of pharmacological and hormonal agents with known mechanisms of action to determine whether differences in response exist between those agents that work directly upon the muscle and those that work indirectly through a neural effect.

METHODS

Studies were carried out in four groups of patients. The first group consisted of 10 patients, aged 22-56 (mean, 42) with Raynaud's disease. Classic Raynaud's phenomenon was defined as intermittent pain or paresthesias of symmetrical digits on emotional stress or exposure to cold with the sequential development of the characteristic color changes of pallor, cyanosis, and then rubor (8, 9). Incomplete Raynaud's phenomenon was defined as intermittent development of one of the characteristic color changes on emotional stress or exposure to cold with or without the pain or

¹ Abbreviation used in this paper: LES, lower esophageal sphincter.

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paresthesias. Six patients had classic while four patients had incomplete Raynaud's phenomenon. Patients in this group had no clinical or serological evidence of scleroderma, polymyositis, systemic lupus erythematosus, or rheumatoid arthritis. The second group consisted of 22 patients aged 35-59 (mean, 49) with scleroderma as defined as diffuse waxy induration of the skin with fixation to the underlying tissue (10) (Table I). All patients with scleroderma had Raynaud's phenomenon, 10 classic and 12 incomplete. A third group consisted of 20 patients, aged 44-76 (mean, 62) with isolated LES incompetence. Criteria for selection was an LES pressure below 8 mm Hg. The fourth group consisted of 20 normals, aged 22-76 (mean, 44). None of the patients had undergone upper gastrointestinal tract surgery.

All patients were studied while resting quietly in the supine position after an 8 hr fasting period. Belt pneumographs around the chest and over the larynx were used to monitor respiration and swallowing, respectively. Three water-filled polyvinyl catheters, 1.4 mm internal diameter, were used to transmit intraluminal pressures to external transducers (Statham P23BB). Output from these transducers was recorded on a multichannel Beckman curvilinear ink-writing recorder. The recording catheters were arranged to measure intraluminal pressure at three points, 5 cm apart, through side openings 1.4 mm in diameter. The pressure recording tips were infused with distilled water by a syringe pump at a constant rate of 2.0 ml/min. LES pressures were recorded as millimeters of mercury with mean gastric fundal pressure used as the zero reference. The manometric records were tabulated using the mid-respiratory pressure recorded from the segment of the LES manifesting the maximal pressure. The recording assembly was positioned with all orifices in the stomach. After a 20 min rest period, the assembly was moved at 1.0 cm intervals through the full length of the esophagus. After this diagnostic evaluation, the recording assembly was positioned and anchored so that pressures were recorded simultaneously from the esophagus, LES, and stomach.

TABLE I
Criteria for Scleroderma*

Absolute
Characteristic cutaneous lesions involving more than just the digits
Additional
History of classic Raynaud's phenomenon
Diffuse fibrosis on chest X-ray or destruction of distal phalanges on hand X-rays
Restrictive changes or decreased diffusing capacity on pulmonary function tests
Cor pulmonale or conduction defect on electrocardiogram
Exclusions
Electromyogram or muscle biopsy changes of polymyositis
Characteristic joint involvement of rheumatoid arthritis
Positive LE prep or clinical systemic lupus erythematosus
Periarteritis nodosa by biopsy or clinical course
Typical rheumatoid nodules
Latex titer 1:160 greater
Proximal muscle weakness

* Absolute and one additional required for diagnosis.

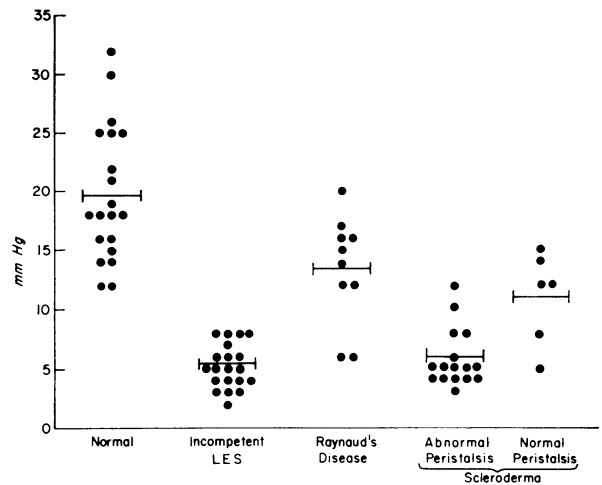


FIGURE 1 Basal lower esophageal sphincter pressure for all subjects and patients. Each point represents the mean value obtained from the three recording orifices.

Gastrin I, amino acid sequence 2-17 (Hexadecapeptide Amide),² was given intravenously as a single 30 sec injection through an indwelling antecubital catheter. Edrophonium chloride (Tensilon)³ was given intravenously over a 30 sec period. Methacholine chloride (Mecholyl)⁴ was given subcutaneously. Each compound was administered at the lowest dose that gave the maximal response in each group of patients. Injection of each agent was either given on separate days or were given on the same day with a 1 hr interval between injections. Informed consent was obtained in all subjects and patients. Statistical analysis was performing using Student's *t* test.

Initially, five normal subjects were studied to ascertain whether the mechanism of action of gastrin I in man was similar to that in the opossum (11). Intravenous infusion of atropine sulfate at 12 $\mu\text{g}/\text{kg}/\text{hr}$ was administered at a constant rate. This rate of infusion did not alter baseline LES pressure. Maximal doses of methacholine (40 $\mu\text{g}/\text{kg}$) and gastrin I (0.5 $\mu\text{g}/\text{kg}$) were tested before and during the atropine infusion. In the presence of atropine, the LES response to methacholine was significantly reduced to $8.3 \pm 3.7\%$ of its control value ($P < 0.001$). The response to gastrin I was similarly reduced to $12.4 \pm 4.6\%$ of its control value ($P < 0.001$). These data indicate that in man, as in the animal model, gastrin I is antagonized by atropine, which suggests that gastrin I is acting through acetylcholine release.

RESULTS

In Fig. 1 are shown the LES pressures recorded in 22 patients with scleroderma, 10 patients with Raynaud's disease, the control groups of 20 normals and 20 patients with an incompetent LES. The patients with scleroderma are divided into two groups: (a) six patients with nor-

² Imperial Chemical Industries Ltd., Alderley Park, Cheshire, England.

³ Roche Laboratories, Div. Hoffmann-LaRoche Inc., Nutley, N. J.

⁴ Merck & Co., Inc., Rahway, N. J.

TABLE II
Lower Esophageal Sphincter Pressure; Basal Level and Peak Response to Each Agent

	Basal LES	Peak LES response to methacholine (40 μ g/kg)	Peak LES response to edrophonium (80 μ g/kg)	Peak LES response to gastrin I (0.5 μ g/kg)
	mm Hg	mm Hg	mm Hg	mm Hg
Normals	19.4 \pm 1.3	38.8 \pm 6.0	33.2 \pm 4.3	87.7 \pm 4.1
Scleroderma (normal peristalsis)	11.0 \pm 1.6	21.4 \pm 3.6	17.6 \pm 2.6	22.1 \pm 2.8
Scleroderma (abnormal peristalsis)	5.8 \pm 0.6	13.1 \pm 1.2	2.6 \pm 0.2	2.9 \pm 0.3
Raynaud's disease	13.4 \pm 1.4	26.5 \pm 3.7	23.4 \pm 2.7	26.8 \pm 3.4
Incompetent LES	5.3 \pm 0.4	11.5 \pm 1.2	11.4 \pm 1.7	22.3 \pm 0.5

Each value represents the mean \pm SE. The peak LES response is the change in pressure from basal level.

mal distal peristalsis, defined as propulsive esophageal contractions with a mean peristaltic amplitude of over 30 mm Hg; and (b) 16 patients with abnormal distal peristalsis, defined as esophageal contractions with a mean amplitude of less than 5 mm Hg with or without aboral progression. In patients with scleroderma with abnormal peristalsis, 12 had absence of contractions in the distal esophagus and four had contractions of low amplitude. All but one patient with Raynaud's disease had normal distal peristalsis. All normals and patients with an incompetent LES had normal peristalsis. Each point represents the mean LES pressure obtained during the pull-through of the three recording orifices. The LES pressure in normals, 19.4 \pm 1.3 mm Hg (mean \pm SE), differed significantly from the mean levels obtained in each of the other groups ($P < 0.1$). The LES pressure in patients with scleroderma with normal peristalsis 11.0 \pm 1.6 mm Hg, did not differ significantly from the value of 13.4 \pm 1.4 mm Hg obtained in patients with Raynaud's disease. The LES pressure in these groups differed from the value

of 5.8 \pm 0.6 mm Hg obtained in patients with scleroderma with abnormal peristalsis ($P < 0.01$). The group of patients selected for low LES pressure had a mean value of 5.3 \pm 0.4 mm Hg.

In Fig. 2 are shown the LES responses of all patients to subcutaneous methacholine. The lowest dose that gave the maximal response (40 μ g/kg) was used. The response was expressed as a percent increase above the baseline LES pressure (12, 13). The absolute rise in LES pressure in mm Hg is listed for all patients in response to all agents in Table II. The per cent change in LES pressure in response to direct muscle stimulation by a parasympathomimetic agent (14, 15) was not statistically different when each of the groups was compared with normals ($P > 0.05$). All but two patients with scleroderma showed a response to methacholine that was near the mean value. These two patients with scleroderma and abnormal peristalsis did not respond with a significant increase in LES pressure on repeated occasions to higher and lower doses of methacholine. They will be further discussed.

Since the LES in all groups showed a statistically similar per cent response to direct stimulation by methacholine, it seemed possible that agents that acted indirectly through intact cholinergic nerves might distinguish differences among these groups of patients. To determine the response of the LES to endogenously released acetylcholine, the LES pressure was tested to a cholinesterase inhibitor, edrophonium chloride (Fig. 3) (14, 15). Edrophonium enhances the effect of released acetylcholine by inhibiting its breakdown and would only act in the presence of local acetylcholine (14, 15). The maximum per cent response of the LES in patients with scleroderma with abnormal peristalsis was less than that recorded in each of the other groups ($P < 0.001$). The maximum per cent response of the LES in patients in the other three groups did not differ from normals ($P > 0.05$).

To further test cholinergic nerve function in these patients, the LES pressure was evaluated during intrave-

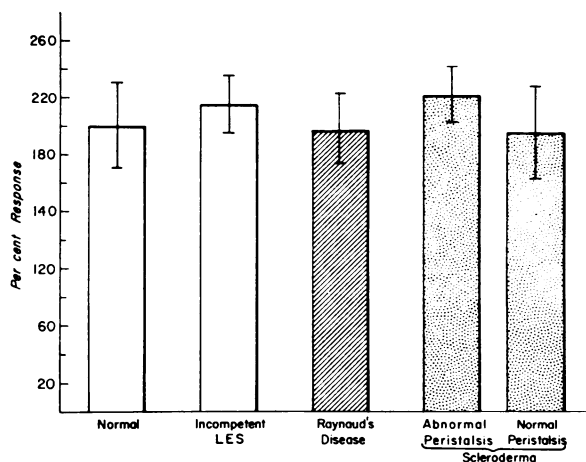


FIGURE 2 Lower esophageal sphincter response to methacholine chloride (40 μ g/kg) for all subjects and patients. The mean \pm SE of the per cent response is shown. There was no statistical difference between each of the groups.

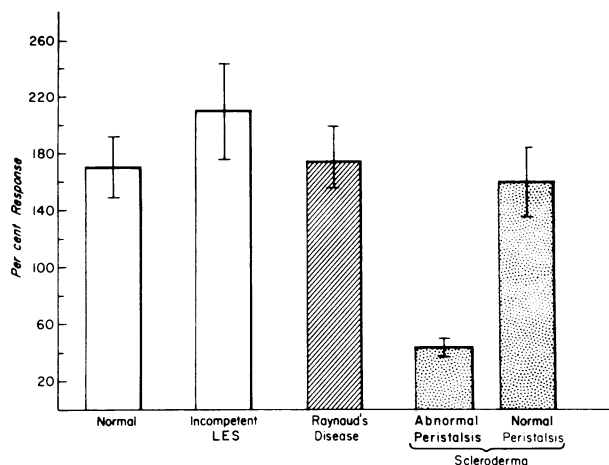


FIGURE 3 Lower esophageal sphincter response to edrophonium chloride ($80 \mu\text{g}/\text{kg}$) for all subjects and patients. The mean \pm SE of the per cent response is shown. Patients having scleroderma with abnormal peristalsis differed from each of the other groups ($P < 0.001$). Other groups did not differ from normals.

nous administration of gastrin I. Each patient received the dose that gave the near maximal response in normals, $0.5 \mu\text{g}/\text{kg}$. In addition, some patients in each group received multiple doses of gastrin I. In Fig. 4, the LES response to gastrin I is plotted against the full dose response curve obtained in normal subjects. Patients having scleroderma with abnormal peristalsis showed a markedly diminished response to all doses of gastrin I. The patients having scleroderma with normal peristalsis and the patients having Raynaud's disease also showed a decreased response, but to a lesser degree. However, each of these groups responded significantly less than normals ($P < 0.001$), but their response was greater than the response in patients with scleroderma and abnormal peristalsis ($P < 0.001$). Patients with isolated LES incompetence responded to the same degree as did normals.

When these pharmacological data were correlated with the manometric findings in patients with scleroderma and Raynaud's disease, three groups of patients could be distinguished. Group one included all patients whose manometric evaluation revealed normal peristalsis. In this group were all but one patient with Raynaud's disease and six patients with scleroderma. All patients in group one had a minimal reduction in LES pressure, a normal response to methacholine and edrophonium, and a partially diminished response to gastrin I. Patients in group two had abnormal peristalsis with a normal LES response to methacholine. This group consisted of 14 patients with scleroderma and one patient with Raynaud's disease. These patients had a marked reduction in LES pressure and their response to gastrin I and edrophonium was greatly reduced. Patients in group three were char-

acterized by both abnormal peristalsis and a failure of the LES to respond to methacholine, gastrin I, and edrophonium. Two patients with scleroderma were in this group. In Table III, features of the illness were compared in the three groups of patients. Neither the presence of classic or incomplete Raynaud's phenomenon nor the duration of the disease corresponded with the groupings. Although both patients in group three had scleroderma for more than 8 yr duration, patients in the other groups had Raynaud's phenomenon and scleroderma for equal periods. Neither patient in group three had an esophageal stricture while several patients in group two and in the incompetent LES group had esophageal strictures.

DISCUSSION

Pharmacological agents with known sites of action have been used extensively to evaluate the response of in vitro gastrointestinal smooth muscle to either direct muscle or indirect neural stimulation (14). This technique has not been generally utilized to evaluate the mechanism of gastrointestinal motor dysfunction in vivo. The purpose of this study was to determine the LES response to compounds with different mechanisms of action in patients with well-defined clinical disease. The LES was evaluated because it provided an accessible area of smooth muscle frequently involved in scleroderma (1-7). In addi-

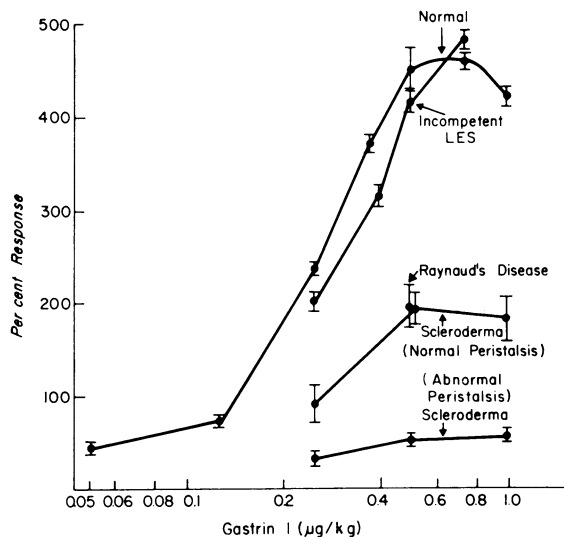


FIGURE 4 Lower esophageal sphincter response to gastrin I. The mean \pm SE of the per cent response is shown. The dose of $0.5 \mu\text{g}/\text{kg}$ was administered to all subjects and patients. Complete dose response curves were obtained in 13 normals, three patients having scleroderma with normal peristalsis, seven patients having scleroderma with abnormal peristalsis, and five patients with an incompetent LES. The maximal LES response at $0.5 \mu\text{g}/\text{kg}$ gave clear separation of individual values between all patients with scleroderma having normal peristalsis, normals and all patients with scleroderma having abnormal peristalsis.

TABLE III
Comparison of Manometric and Pharmacological Groups to Duration of Illness

	Raynaud's phenomenon		Duration of Raynaud's phenomenon		Duration of scleroderma	
	Classic	Incomplete	Range	Mean \pm SE	Range	Mean \pm SE
Group I: normal peristalsis						
Raynaud's disease (9)	4	5	1-7	3.7 \pm 0.8	—	—
Scleroderma (6)	4	2	1-30	4.6 \pm 8.6	1-6	3.2 \pm 0.8
Group II: abnormal peristalsis and normal response to methacholine						
Raynaud's disease (1)	1	—	5	—	—	—
Scleroderma (14)	5	9	1-26	7.1 \pm 2.8	1-11	4.3 \pm 1.2
Group III: abnormal peristalsis and diminished response to methacholine						
Scleroderma (2)	1	1	12-16	14	8-13	10.5

tion, the LES response to different agents could be readily quantified (12, 13). The three compounds used in this study were chosen because they have well-defined mechanisms of action and could be administered to man (14, 15). Methacholine, a parasympathomimetic, was chosen to provide an index of direct muscle response. Edrophonium, a cholinesterase inhibitor, and gastrin I, a hormone that works through acetylcholine release, were utilized to provide a measure of cholinergic nerve function. Utilization of gastrin I also allowed us to evaluate the LES response to the hormone that has been shown to be the major determinant of resting LES competence (16). The expression of the data as a percent increase above basal LES pressure allowed us to normalize the responses and make comparisons between relative changes in each group. Since control groups with normal and reduced LES pressures were utilized, absolute pressure changes could also be compared with similar results.

These studies demonstrated several findings. First, gastrin I, which acted through acetylcholine release, gave a reduced LES response in all patients with scleroderma and Raynaud's disease, including those with normal peristalsis. Second, in those patients with abnormal peristalsis and a diminished LES pressure, there was a failure to respond to a cholinesterase inhibitor (edrophonium) which acted to enhance the effect of local acetylcholine. Third, except in two patients with scleroderma (group three), the LES responded by the same per cent increment as did normals when stimulated directly by a parasympathomimetic agent (methacholine). These findings indicated that an abnormality in the response to compounds acting indirectly through cholinergic nerves was present at a time when the response to muscle stimulation by a cholinergic compound was preserved.

When these pharmacological data and the manometric findings were combined, three groups of patients could

be distinguished (Table III). The findings in these three groups were used to explain several isolated clinical observations available in the literature. Patients in group one had normal peristalsis and a moderately reduced response to gastrin I. This initial abnormality in response to an indirect acting compound, gastrin I, may explain the close relationship of esophageal dysfunction and Raynaud's phenomenon, even in the absence of scleroderma. These data suggested that all patients with Raynaud's phenomenon, either as an isolated finding or with scleroderma, have a latent abnormality in esophageal function. A close clinical association between Raynaud's phenomenon and esophageal dysfunction has previously been reported (2). The preserved response to both methacholine and edrophonium in this group suggests both intact muscle response and acetylcholine release. The diminished response to gastrin I suggests that latent cholinergic nerve dysfunction may be demonstrated only when these nerves are directly stimulated.

Patients in group two had abnormal peristalsis and a markedly diminished LES pressure. They responded with a normal per cent increment in LES pressure to methacholine. The postmortem examination of patients previously evaluated by manometry demonstrated normal smooth muscle by light microscopy at areas earlier shown to be markedly abnormal in performance (1). This finding suggested that either neural dysfunction preceded the changes in the muscle or that primary muscle function was abnormal before detectable pathologic changes by light microscopy. The difference in response in patients in group two between a direct muscle stimulus, methacholine, and compounds that acted indirectly through cholinergic nerves, edrophonium and gastrin I, suggested a defect in neural function. This finding did not eliminate the possibility of a coexistent primary muscle abnormality as well, but simply indicated that muscle elements could

respond to a greater degree when stimulated directly than when stimulated indirectly. This group of patients with marked esophageal dysfunction but preserved muscle response to methacholine may be the group that have been reported to improve after intra-arterial reserpine (4).

In group three, patients had abnormal peristalsis and an absent response to direct muscle stimulation by methacholine. As previously reported, at postmortem examination, the predominant smooth muscle abnormality in the esophagus was atrophy (1, 7). The absent response to direct cholinergic muscle stimulation suggested muscle failure. These patients in group three may be at a phase in their disease where muscle atrophy was present similar to that reported in patients dying with scleroderma. In support of this hypothesis, a single patient, W. K., was found to have abnormal peristalsis and an absent response to methacholine. At autopsy there was marked smooth muscle atrophy of the esophagus.

While the data in this study implied that in patients with scleroderma and Raynaud's disease, a defect in neural function may be responsible for some portion of the esophageal manifestations of these diseases, the pathogenesis of this neural dysfunction was not clear. Several mechanisms could explain these findings. First, the changes in neural and subsequent muscle function may be caused by alterations in blood flow due to esophageal vasoconstriction associated with the Raynaud's phenomenon. Second, the adrenergic dysfunction that produces Raynaud's phenomenon might suppress cholinergic function through recently described interconnections of these neural systems (17-20). Third, a basic process might cause esophageal dysfunction and the other manifestations of the disease. The data in this study did not determine the mechanism responsible for neural dysfunction in patients with scleroderma and Raynaud's disease.

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