Electrical stimulation of esophageal smooth muscle and effects of antagonists

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METHODS

Adult opossums were anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg) and the esophagus was excised. Muscle strips, 1 X 2 cm, were cut from 4–8 cm above the gastroesophageal junction. Longitudinal strips, with the mucosa removed, displayed activity chiefly of the longitudinal layer of the muscularis propria. Transverse strips displayed activity of the circular layer of the muscularis propria. Separate strips of mucosa were cut longitudinally to study actions of the muscularis mucosa whose cells are oriented mostly longitudinally. The strips were vertically mounted in separate 50-ml baths of Krebs solution bubbled with 95% O₂-5% CO₂ at 36–37°C, and were attached by fine silver chains to force-displacement transducers (Grass Instrument Co., model FT.03C), at a basal tension of 2 g. Tension changes were displayed on an ink-writing polygraph (Beckman type R Dynograph). The Krebs solution contained (mm): Na⁺ 138.5, K⁺ 4.6, Ca²⁺ 2.5, Mg²⁺ 1.2, Cl⁻ 125, HCO₃⁻ 21.9, P.O₄⁻ 1.2, SO₄²⁻ 1.2, glucose 49.3. The tissue was stimulated with two fine stainless steel electrodes, 5 mm apart, which pierced each strip near the fixed end. The anode was nearest the fixed end of the strip except where the reverse position is stated in the RESULTS. A square-wave stimulator (Grass Stimulator model S8, with stimulus isolation unit SIU 46/8) delivered 10-sec trains of monophasic square waves at intervals of about 1 min. Square-wave frequency, voltage, and duration were varied as described in the RESULTS. Voltage calibration represents direct-current voltage outputs of the stimulator measured before each experiment. The following drugs, dissolved in distilled water, were added to each bath as necessary for the experiments: acetylcholine bromide, atropine sulfate, carbamylcholine chloride, physostigmine sulfate, hexamethonium bromide, methysergide maleate, nicotine sulfate, propranolol hydrochloride, tolonaline hydrochloride, tetrodotoxin, and D-tubocurarine chloride. All drugs were added in volumes of less than 1 ml to achieve bath concentrations expressed as grams per milliliter of the base. Before a drug was added...
to the bath, three or more control responses were recorded to stimuli delivered at 1-min intervals. Test responses were the first three or more responses to identical stimuli delivered at least 5 min after adding the drug. Responses were graded by measuring the maximal amplitude of contractions.

RESULTS

Longitudinal Layer of Muscularis Propria and Muscularis Mucosa

Longitudinal strips of muscularis propria, with mucosa removed, and longitudinal strips of muscularis mucosa were seldom spontaneously active. Effective electrical stimulation of these strips always produced the duration response, a sustained contraction throughout the stimulus period. Square-wave frequency, voltage, and pulse duration were varied independently to obtain optimal stimulus characteristics for the response. Pulse durations of less than 0.1 msec were ineffective. The amplitude of response was nearly maximal with pulses of 1.0 msec; we used 1.0-msec pulses because longer pulses are likely to damage tissues. Responses of near maximal amplitude occurred at 20–30 v. As frequency increased from 1 cycle/sec, the response amplitude increased to become maximal at 40–50 cycles/sec, and gradually diminished at higher frequencies. Single shocks produced no response. The amplitude at any given frequency was slightly greater if the frequency was increased rather than decreased. Also, amplitudes slightly increased as the strip aged. These factors account, in part, for the variance of the frequency response curve shown in Fig. 1.

To examine the effects of drugs on this response, we used 10-set trains of square waves of 30 v, 1.0 msec, 45 cycles/set, delivered at 1-min intervals. Tetrodotoxin, 10⁻⁷ g/ml, abolished the duration response; the effect was dose related (Fig. 2). Hexamethonium, 10⁻⁴ g/ml, and nicotine, 10⁻⁴ g/ml, had no effect.

In the outer longitudinal muscle strips, atropine delayed the onset of contractions, reduced their amplitude, and retarded relaxation; the effect was dose related (Figs. 3, 4A and 4B). Atropine antagonism to contractions was greatest and often complete during the first 8–10 sec of the stimulus period. If a train was prolonged more than 10 sec, a contraction developed or progressed to a height which was usually less than controls. Relaxation following the end of a prolonged stimulus was delayed in onset and
gradual. Another stimulus train delivered during the period of prolonged relaxation facilitated the relaxation (Fig. 4A). The effects of atropine on the mucosal muscle were similar except that atropine tended to retard the rate of contraction of mucosal muscle rather than to delay the onset of contraction as observed for outer longitudinal muscle (Fig. 4B).

d-Tubocurarine, $10^{-4}$ g/ml, had no consistent effect on either duration response. Propranolol, $5 \times 10^{-4}$ g/ml, had no effect, but concentrations of $10^{-4}$ g/ml depressed the duration response. Toluazoline, $5 \times 10^{-4}$ g/ml, either had no effect on the duration response or slightly potentiated it; higher concentrations caused spontaneous contractions not opposed by atropine, $10^{-6}$ g/ml, or tetrodotoxin, $10^{-5}$ g/ml. Methysergide, $10^{-5}$ g/ml, had no effect.

Circular Layer of Muscularis Propria

Transverse strips showed no spontaneous activity. Electrical stimulation could elicit two responses in this muscle layer. A brief contraction, the off response, usually occurred after the end of the stimulus. A similar response occasionally occurred at initiation of the stimulus, the on response. They were examined separately.

Off response. The electrical stimulus parameters were varied independently to obtain optimal stimulating conditions. The response to varying pulse duration was similar to that of the longitudinal strips; a 1.0-msec duration was used. A maximally effective voltage was usually between 15–20 v, but a 30-v stimulus was usually used. The amplitude of the response varied with the frequency.

Single shocks produced no response. As frequency increased from 1 cycle/sec, the amplitude increased sharply to reach a maximum at 10 cycles/sec, diminished sharply at higher frequencies, and was often absent above 40 cycles/sec (Fig. 1). As in longitudinal muscle, the response amplitude varied with the frequency to which the strip had been previously exposed. The variation of amplitude, however, was greater for the off response than for the duration response.

To measure the effects of drugs on this response, we used 10-sec trains of square waves of 30 v, 1.0 msec, 10 cycles/sec, delivered at 1-min intervals. Tetrodotoxin, $10^{-7}$ g/ml, abolished the off response and the effect was dose related (Fig. 2). Neither hexamethonium, $10^{-4}$ g/ml, nor nicotine, $10^{-2}$ g/ml, affected the off response. Atropine did not block this response (Figs. 3, 4C, and 5), though it was sometimes depressed at $10^{-6}$ g/ml (Fig. 5). Higher concentrations of atropine potentiated the off response (Figs. 3 and 4C). Atropine, $10^{-8}$ g/ml, abolished contractions produced by carbachol, $10^{-7}$ g/ml, and acetylcholine, $10^{-6}$ g/m, in the
presence of physostigmine, 10^{-7} g/ml (Fig. 5). d-Tubocurarine, 10^{-5} g/ml, had no effect on the off response. Propranolol, 5 \times 10^{-6} g/ml, had no effect but the response was depressed at concentrations of 10^{-4} g/ml. Tolazoline, 5 \times 10^{-6} g/ml, either had no effect or potentiated the off response; higher concentrations caused spontaneous contractions not antagonized by atropine or tetrodotoxin. Methysergide, 10^{-5} g/ml, had no effect.

**On response.** The on response was difficult to study because it did not occur consistently. It was rarely observed at 10 cycles/sec, 1 msec, 30 v, particularly after the first few stimulus periods. An on response, however, always occurred with direct-current stimulation or square waves at very high frequencies if the anode was nearer the lower fixed layers than did such tubular organ preparations as the Trendelenberg preparation and the whole esophagus preparation we have previously described (8). The electrodes implanted in the fixed end of the strips did not greatly hinder contractions, and the contractions involved the entire strip.

**Discussion**

Study of the autonomic innervation of esophageal smooth muscle requires techniques to selectively stimulate the various motor nerves. In some organs the nerves may be selected by dissection as in the Finkleman preparation of the duodenum (9) and the preparations of colon described by Garry and Gillespie (10, 11). Postganglionic sympathetic motor nerves generally reach these viscera separate from the motor nerves of craniosacral origin. In the esophagus, however, the sympathetic and parasympathetic nerves mingle in the esophageal plexus before they enter the muscle wall and the sympathetic nerves reach the plexus in numerous delicate strands (22). Thus, it would be difficult to achieve discriminate stimulation of extrinsic nerves.

Indiscriminate stimulation of intramural nerve elements is produced by transmural stimulation as described by Paton (24), and the techniques used by Bennett (2) and Campbell (4). We found, however, that the paired intramural electrodes described in methods produced satisfactory results. Field stimulation did not work. Isolated strips permitted a clearer separation of the functions of the muscle layers than did such tubular organ preparations as the Trendelenberg preparation and the whole esophagus preparation we have previously described (8). The electrodes implanted in the fixed end of the strips did not greatly hinder contractions, and the contractions involved the entire strip.

Electrical stimulation of this preparation may excite both local inhibitory and excitatory nerves as well as act directly on the muscle cells. The responses observed under the various conditions must be viewed as the net result. Tetrodotoxin is considered to block nerve conduction without affecting smooth muscle directly (18). The ability of tetrodotoxin to eliminate the off response of circular muscle and
the duration responses of both longitudinal and mucosal muscle indicates that these responses are nerve mediated. The on response of circular muscle produced by direct current stimulation even in the presence of tetrodotoxin appears, therefore, to result from direct muscle stimulation. Some autonomic nerves, however, may be resistant to the effects of tetrodotoxin (12).

Hexamethonium and nicotine did not affect the nerve-mediated off and duration responses. These responses, therefore, are due to excitation of postganglionic elements of the neural plexuses. The absence of antagonism to these responses by tolazoline and propranolol indicate that adrenergic nerves acting upon adrenergic alpha and beta receptors are not involved. The depression of responses by large concentrations of propranolol can be attributed to the local anesthetic properties of this drug (21). The potentiation of these responses and the excitation produced by large concentrations of tolazoline suggest that there may be excitatory adrenergic alpha receptors in this tissue. Excitatory alpha receptors have been identified in esophageal smooth muscle in the opossum (unpublished data) and in other species (5, 6). The excitation produced by tolazoline is not nerve mediated since tetrodotoxin did not alter this action. Since methysergide did not alter the responses to electrical stimulation, serotonin apparently is not directly involved.

The duration responses of both longitudinal and mucosal muscle are antagonized by atropine, indicating that these responses involve cholinergic mechanisms acting on muscarinic receptors. Prolonged electrical stimulation, however, could overcome the effect of atropine, and atropinized strips excited by prolonged stimulation were slow to relax. A delayed atropine-resistant response to vagal stimulation in longitudinal esophageal smooth muscle of the chicken was reported recently by Hassan (13).

It was surprising that atropine did not obliterate the off response of circular muscle. It is likely that atropine could penetrate to the circular layer in sufficient concentration to block cholinergic mechanisms acting on effector cells.(19). Alternatively, excitation of inhibitory nerves may contribute to the off response of the smooth muscle under these conditions. The depression of responses and the excitation produced by large concentrations of tolazoline may be due to this effect.

In conclusion, the duration responses of the mucosal and longitudinal muscle appear to be mediated by similar mechanisms. They exhibit similar pharmacological and optimal stimulus characteristics. Both have an atropine-sensitive and an atropine-resistant component that are nerve mediated. The off response of the circular muscle is mediated by nerves which may be either noncholinergic motor nerves or nonadrenergic inhibitory nerves. The on response observed in circular muscle, produced by direct-current stimulation, appears to result from a direct effect on the muscle; the response was not blocked by any of the drugs used. A different and nerve-mediated on response, however, may also be present since early in experiments an on response was occasionally seen at the low frequencies of 10 cycles/sec irrespective of electrode polarity.

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