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Oxygen Dependence of Contractions in Esophageal and Gastric Pyloric and Ileocecal Muscle of Opossums (41418)

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Abstract. Strips of muscularis propria were so cut that contractions would represent actions of smooth muscle of longitudinal and circular layers of esophageal body and circular layer of esophagogastric sphincter, oblique layer of gastric fundus and circular layer of pylorus and ileocecal junction. Isometric tone, a force maintained in the absence of stimulation, was present only in fundus and sphincter strips. Strips from esophageal body contracted in response to electrical field stimulation of intramural nerves: in longitudinal muscle these responses were atropine sensitive; in circular muscle they were not. Oxygen dependence was tested by equilibrating bath solutions with gas mixtures in which nitrogen incrementally replaced oxygen, and by exposing strips to metabolic inhibitors. Isometric tone in all sphincter and fundus muscles declined as oxygen was replaced with nitrogen. It was abolished by metabolic inhibitors. Neurogenic contractions of both layers of esophageal body muscle and the contractions induced by carbachol, histamine, and substance P in sphincter strips were not oxygen dependent. Thus, isometric tone in gastrointestinal muscle differs from other kinds of contractions in its requirement for oxygen.

Isometric tone, a stable force that is maintained in the absence of stimulation, characterizes those regions of the gut that are called sphincters. Certain drugs seem to selectively affect this tone as compared to rhythmic contractions (1). This tone can be easily measured in sphincter muscle by measuring the depth of relaxation that is induced by maximal stimulation of intrinsic inhibitory nerves.

In the esophagus one can compare those muscles that are characterized by such tone with those muscle segments that are not. In the smooth muscle part of the esophagus, the circular muscle layer of the esophageal body shows no such tone. The esophagogastric sphincter, in contrast, is characterized by a high degree of isometric tone (2, 3). Such isometric tone has also been described in muscle cut from the gastric fundus, the pylorus, and the ileocecal junction (4–6).

The stable tone that characterizes muscle from the esophagogastric sphincter is myogenic (7). Myogenic tone has also been described in other smooth muscles, where it has been observed to diminish with hypoxia. The ability of hypoxia to depress myogenic tone has been studied in a variety of muscle preparations: in rat and cat uterus (8), in the small intestine of guinea pig, rat, and rabbit (8–10), cat colon (10), guinea pig taenia coli (11, 12), in aortic rings and strips (13–17), and in isolated tibial arteries (18). This effect of hypoxia could represent a reduction in the synthesis or the action of intramural prostaglandin (19).

In those parts of the gut where myogenic tone is most prominent, sphincteric regions, this effect of hypoxia has not been studied. Thus, the myogenic tone that characterizes sphincteric areas in the gut was studied to see if it is oxygen dependent. Nerve-induced contractions of the nonsphincteric muscle of the esophagus were compared with the tone of the sphincter in this respect. Tone in the gastric fundus, pylorus, and ileocecal junction was also examined. Also, drug-induced contractions in strips from the lower esophageal sphincter were examined to test the specificity of the effect of hypoxia.

Methods. Mature opossums of either sex

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were anesthetized by intracardiac injection of sodium pentobarbital, 40 mg/kg. Opossums are very resistant to anesthetic agents and this is very nearly the lethal dose. The abdomen was opened and the organ to be studied was excised, opened, and transferred to a 1-liter bath of Krebs solution equilibrated with 95% O_2–5% CO_2 at 37°. The mucosa was removed and the muscular wall was cut into strips about 1 cm long, 2 mm wide, and 1 mm thick in the relaxed state. The Krebs solution had the following composition: sodium, 138.5 m*M;* potassium, 4.6 m*M;* calcium, 2.5 m*M;* magnesium, 1.2 m*M;* chloride, 125.0 m*M;* phosphate, 1.2 m*M;* bicarbonate, 21.9 m*M;* glucose 11.5 m*M*. Its pH, when it was bubbled with gas mixtures containing 5% CO_2, was 7.40.

In experiments designed to study esophageal muscle, three kinds of strips were prepared. Some strips were cut in the long axis of the organ, *longitudinal strips.* Tension recorded from such strips reflects the activity of the outer longitudinal muscle layer of the esophageal body. Other strips were cut transversely from the esophageal body, *transverse body strips.* Tension recorded from such strips reflected the activity of the inner circular muscle layer. Other strips were cut transversely from the esophagogastric junction, *transverse sphincter strips.* Tension recorded from these strips reflected activity of the inner circular muscle layer of the esophagogastric sphincter.

These three kinds of strips were transferred to a 1.5-ml superfusion bath in which their tension could be continuously recorded in a flowing stream of Krebs solution equilibrated with 95% O_2–5% CO_2 at 37°. Each strip was elongated to its length of maximum tension development when stimulated electrically to contact supramaximally. In the case of sphincter strips, the length of optimal tension development was determined with supramaximal concentrations of acetylcholine, 10^{-6} *M*. In all strips, this stretch is to a length that is about 150% of L_0. Under these circumstances, length was more than 1.5 cm, while width and thickness were reduced to less than 0.5 mm. Electrodes in the bath allowed periodic electrical field stimulation of the intramural plexuses (20, 21).

The three kinds of strips behave differently at rest and when excited electrically (2, 3). Sphincter strips, upon stretch, exhibit tone that is myogenic, and relax during the train of pulses (2, 3). Transverse strips from the esophageal body, after the end of the train, contract once, to generate a single slow twitch called the *off-response* (4, 7, 21). Longitudinal strips contract during the stimulus train, the *duration response.* In any single experiment, strips of all three kinds were cut from the same esophagus and treated identically.

In other experiments, strips of the muscular wall were cut transversely from the pylorus and from the ileocecal junction. These strips respond like those of the esophagogastric junction in that they develop myogenic tone with stretch and relaxing neurogenically on electrical field stimulation. In still other experiments, strips were cut from the gastric fundus at such angle and location as to reflect mainly the activity of the oblique muscle layer whose muscle bundles can be seen easily after mucosal removal (5). These strips also behave like the esophagogastric sphincter strips.

The fact that isometric tension of sphincter strips is indeed myogenic and abolished by metabolic inhibition was first tested in sphincter strips. They were superfused with Krebs solution that had been equilibrated with 95% O_2–5% CO_2 at 37 ± 1°. They were stimulated with 10-sec trains of rectangular pulses, 0.5 msec long, at 10 Hz and supramaximal current. Trains were repeated every 30 to 60 sec. Tension was recorded continuously with force-displacement transducers (Grass Instrument Co.) recording on an ink-writing polygraph (Beckman Instruments). The experiments were initiated with slack strips. Then, calibrated rack-and-pinion clamps holding the force displacement transducers were adjusted to retract the transducers to elongate the strips to the point where tension was first recorded. The length of each strip was measured and recorded as L_0. Thereafter, the strips were elongated in 20% increments of L_0 to 300% L_0. With each incremental stretch, tension between stimuli rose to a stable new level, falling during field stimulation. At the end of each experiment, a metabolic inhibitor was added to the superfusing Krebs buffer solution. The inhibitors used were 2,4-dinitrophenol (10^{-4} *M*), sodium iodoacetate (10^{-2} *M*), sodium cyanide (10^{-3} *M*), and sodium azide (10^{-4} *M*). Plots were made of the level of stable basal tension between stimuli and length, and between the tension during neurogenic relaxation and length; also, the tension under the influence of the metabolic inhibitors was compared to that during relaxation.

In other experiments, we examined the effect of oxygen deprivation. After 1 hr of stimulation, the gas bubbling the reservoir of Krebs solution was replaced with one of several other gas mixtures in which the O_2 had been replaced by N_2. The gas mixtures used contained the following proportions of O_2, N_2, and CO_2 (in that order): 95–0–5%, 70–25–5%, 50–45–5%, 30–65–5%, 20–75–5%, 10–85–5%, and 0–95–5%. Gas mixtures were substituted for the 95% O_2–5% CO_2 mixture in no fixed sequence. An effect, when found, was evident within 5 min and a steady state was achieved within 10 min, when the required measurement was made. The exposure of the tissue to one of these other gas mixtures lasted less than 20 min, after which bubbling 95% O_2–5% CO_2 was restored. The effect of the substituted gas mixture on tone was fully reversed within 5 min on restoration of the control gas mixture.

Since the bath had a very small surface exposed to the atmosphere and since the bubbling was continuous and vigorous, one would expect that the partial pressure of oxygen would correspond to that of the gas mixture. Indeed, as measured by oxygen polarimetry, actual partial pressures corresponded exactly to the gas mixtures. That is, they were, 95, 70, 50, 30, 20, 10, and 0% during perfusion with the solutions bubbled with gas mixtures containing these proportions of oxygen. Also, pH was identical in the hypoxic solutions to that in the maximally oxygenated solution.

In other experiments, four muscle strips were cut transversely from the lower esophageal sphincter and set at optimal length in the muscle bath. They were subjected to electrical field stimulation consisting of 10-sec trains of rectangular pulses 0.5 msec long, at 10 Hz and supramaximal strength, 38 V. Trains were repeated every 100 sec. After a 90-min equilibration period, the strips were exposed to a range of concentrations of carbachol, histamine, or substance P, first in Krebs solution equilibrated with 95% O_2–5% CO_2 and then in Krebs solution equilibrated with 95% N_2–5% CO_2. Freshly prepared stock solutions of the drugs were injected into the stream superfusing the tissue. Drug concentrations achieved in the stream were calculated from the known dilution determined with phenol red. Strips were superfused for 30–45 min with the Krebs solution equilibrated with 95% N_2–5% CO_2 before the drugs were administered the second time. Electrical field stimulation occurred when the response to the drugs was at a maximum. An interval of 10 to 15 min separated injections. Strips were without O_2 for an average of 100 min in each experiment. Responses to all stimuli were compared for the two gas mixtures using the paired *t* test with $P \leq 0.05$ as the level of significance.

Results. The three kinds of strips from the esophagus behaved at rest and responded to electrical field stimulation as described previously (2, 3). In all three kinds of strips, tension was stable at rest.

The studies with metabolic inhibitors in sphincter strips showed that metabolic inhibition could reduce the stable tension between stimulus trains to a level that was almost exactly that reached during stimulus trains. This is illustrated in the tracing shown in Fig. 1. The stable tension between stimulus trains, which increased with elongation of the strips, will be called total isometric tension: that achieved during the

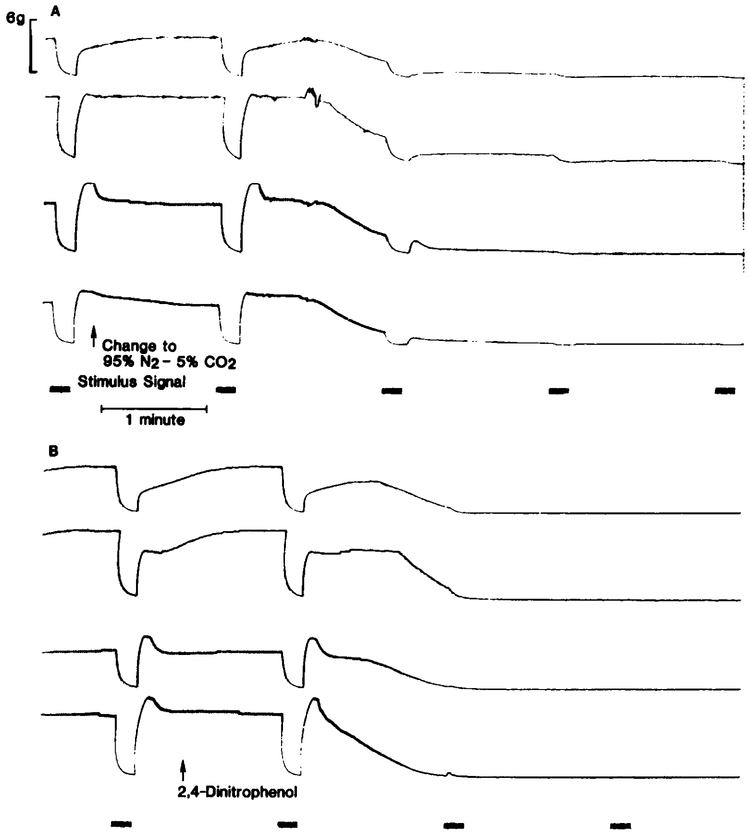

FIG. 1. Panel A is a tracing showing isometric tension in four strips cut from the lower esophageal sphincter. The strips are subjected at regular intervals to electrical field stimulation with trains of rectangular pulses of 0.5-msec duration at 10 Hz and supramaximal current strength, delivered in 10-sec trains at 1-min intervals. As shown, the strips relax. Total isometric tension is the tension above baseline at the initiation of relaxation. Passive tension is that existing at maximal relaxation. At the arrow, oxygen was excluded from the superfusing fluid to reveal the responses in the absence of oxygen. Panel B is a parallel tracing to that shown in panel A. At the arrow, the superfusing solution was changed to one containing 2,4-dinitrophenol, 10^{-4} *M*. Observe that total isometric tension in both cases fell to the level of that achieved during maximal relaxation achieved by electrical field stimulation in the control tracing. The effects of oxygen deprivation and of 2,4-dinitrophenol were fully reversible respectively by restoration of oxygen and by washing.

periods of neurogenic inhibition produced by electrical field stimulation is passive isometric tension. The difference between these is active isometric tension.

Graded degrees of oxygen deprivation affected the three kinds of strips differently. In the longitudinal strips, the duration response produced by electrical field stimulation was depressed but not abolished: even in the solution equilibrated with 95% N_2–5% CO_2, this contraction still occurred with an amplitude about 30% of that found in 95% O_2–5% CO_2. The off-response of the transverse strips of the esophageal body was also depressed but not abolished, even in the solution equilibrated with 95% N_2–5% CO_2. The active isometric tension of the sphincter strips declined in proportion to the O_2 content of the gas mixture used: in the solution equilibrated with 95% N_2–5% CO_2, tone was abolished so that if the inhibitory nerves were still functioning under these hypoxic conditions, no neurogenic relaxation could be appreciated. These re-

TABLE I. AMPLITUDES OF THE THREE KINDS OF CONTRACTIONS OF ESOPHAGEAL MUSCLE AS INFLUENCED BY OXYGEN AVAILABILITY

Gas mixture used (% O_2 Content)	Duration response of longitudinal layer	Off-response of circular layer	Active isometric tension of sphincteric muscle
95	3.3 ± 0.2[a]	2.6 ± 0.1	1.6 ± 0.1
70	2.6 ± 0.3	1.7 ± 0.2*	1.3 ± 0.3
50	2.8 ± 0.3	1.3 ± 0.3*	1.0 ± 0.2*
30	3.2 ± 0.3	1.1 ± 0.3*	0.7 ± 0.2*
20	2.8 ± 0.6	1.4 ± 0.2*	0.4 ± 0.2*
10	2.3 ± 0.4*	1.0 ± 0.1*	0.2 ± 0.2*
0	1.0 ± 0.2*	1.0 ± 0.4*	0

[a] Results are average tension with the standard error of the mean in grams.
* Indicates significant difference ($P < 0.05$) from control response (at 95% O_2).

sults are summarized in Table I. The active isometric tension of sphincter strips was always fully reversed within 30 min after restoration of O_2 to the equilibrating gas mixture.

2,4-Dinitrophenol (10^{-4} M), sodium azide (10^{-3} M), and sodium cyanide (10^{-3} M) all depressed active isometric tension of the sphincter strips more than they did the off-response of transverse strips from the esophageal body and the duration response of longitudinal strips. The inhibitor of glycolysis, sodium iodoacetate, 10^{-4} M, abolished all contractions. These results are summarized in Table II.

Studies of isometric tension in other regions. The strips from the gastric fundus, pylorus, and ileocecal junction also showed active isometric tension that could be abolished by electrical field stimulation of intrinsic inhibitory nerves, as described previously (4–6). This tension was also oxygen dependent, for it declined in all three regions as the oxygen content of the Krebs solution was reduced (Table III). Also, the active isometric tension in all three regions was abolished by 2,4-dinitrophenol (10^{-4} M), sodium azide (10^{-3} M), sodium cyanide (10^{-3} M), and sodium iodoacetate (10^{-3} M).

Responses of lower esophageal sphincter muscle to excitatory agents and to electrical field stimulation in the presence of O_2 and in its absence. When histamine, carbachol, and substance P were injected into the superfusing stream of Krebs solution equilibrated with 95% O_2–5% CO_2, sphincter strips contracted. The magnitude of this response was dose related. The sphincter strips were then superfused with Krebs solution equilibrated with 95% N_2–5% CO_2 and the tone was abolished. A record from a typical experiment appears in Fig. 2. Injections of carbachol into the superfusing stream in the absence of O_2 produced contractions that did not differ significantly from those produced by carbachol in the presence of O_2. These results are summarized in Fig. 3. With injections of substance P, the maximal and immediately

TABLE II. EFFECT OF SOME METABOLIC INHIBITORS ON RESPONSES OF ESOPHAGEAL SMOOTH MUSCLE TO NERVE STIMULATION

Inhibitor used	Duration response (%) of longitudinal layer	Off-response (%) of circular layer	Active isometric tension (%) of sphincteric muscle
2,4-Dinitrophenol (10^{-4} M)	54[a] ± 13	26 ± 3	4 ± 3.2
Sodium azide (10^{-3} M)	90.2 ± 6	20.0 ± 2.8	8.3 ± 1.6
Sodium cyanide (10^{-3} M)	31.0 ± 8	1.5 ± 0.5	0
Sodium iodoacetate (10^{-3} M)	0	0	0

[a] Values indicate amplitude of contraction as a percentage of the amplitude measured in the absence of the inhibitor. Numbers are averages of 10 studies in each kind of strip.

TABLE III. Amplitudes of the Active Isometric Tension in Muscle of the Gastric Fundus, Pylorus, and Ileocecal Junction as Influenced by Oxygen Partial Pressure

Gas mixture used (% O_2 content)	Gastric fundus	Pylorus	Ileocecal junction
95	1.12 ± 0.18[a]	0.71 ± 0.13	1.62 ± 0.23
70	1.11 ± 0.19	0.59 ± 0.12*	1.65 ± 0.23
50	1.07 ± 0.20	0.53 ± 0.11*	1.47 ± 0.21
30	0.87 ± 0.17*	0.32 ± 0.07*	1.10 ± 0.18*
20	0.64 ± 0.10*	0.18 ± 0.04*	0.98 ± 0.16*
10	0.20 ± 0.04*	0.08 ± 0.02*	0.42 ± 0.10*
0	0.03 ± 0.02*	0	0

[a] Values indicate average tension with the standard error of the mean, in grams. There were 10 studies in each kind of strip.

* Indicates significant difference ($P < 0.05$) from control response (at 95% O_2).

submaximal dose produced contractions of the sphincter strips that did not significantly differ from those produced by the same doses of substance P in the presence of O_2. Histamine, in the absence of O_2, produced contractions of the sphincter strips that were less than those produced by the same doses in the presence of O_2. This can be seen in Fig. 4.

Discussion. The gastrointestinal sphincters are parts of the gut where tonic contraction of the circular muscle layer occludes the lumen. Tonic excitation by intramural nerves has been credited for this contraction, at least in part. But a tone that is myogenic has been shown to exist as well in the esophagogastric sphincter (7). There

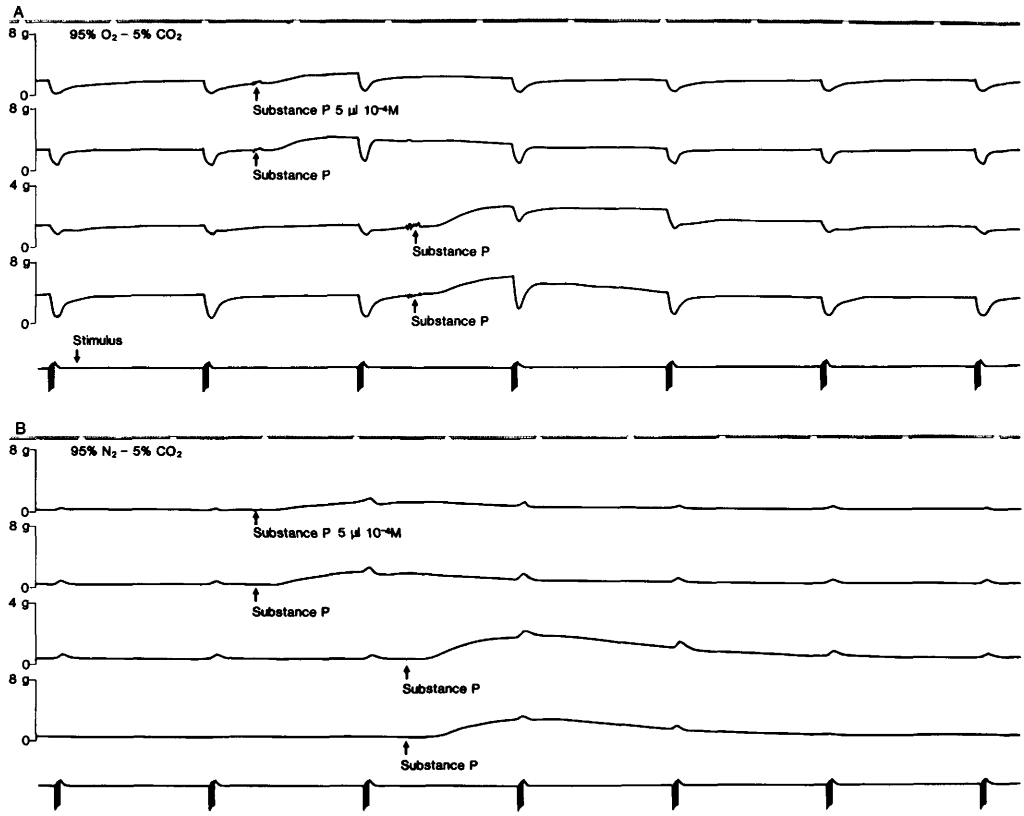

FIG. 2. A record showing sphincter muscle responses to a submaximal dose of substance P, 5 × 10^{-4} *M*, in the presence of oxygen (A) and in its absence (B). The contractions induced by substance P have nearly the same amplitude in both conditions. Note the absence of relaxations in response to electrical field stimulation in hypoxia after substance P has raised the tension of the muscle.

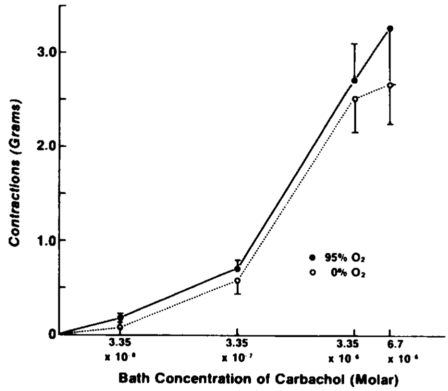

FIG. 3. Carbachol-induced contractions of sphincter muscle as affected by hypoxia. The ordinate shows the amplitude in grams of sphincter muscle contractions above baseline (total isometric tension) caused by histamine. The abscissa is the molar concentration of carbachol in the bath. Closed circles represent sphincter muscle contractions to carbachol in Krebs solution equilibrated with 95% O_2−5% CO_2. Open circles represent the responses of the same strips to carbachol in Krebs solution equilibrated with 95% N_2−5% CO_2. Each point is the mean ± SE for seven strips. The contractions induced by carbachol in the absence of oxygen are not significantly different from those contractions induced by carbachol in the presence of oxygen.

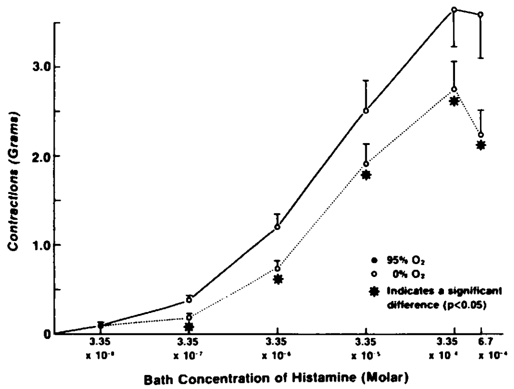

FIG. 4. Histamine-induced contractions of sphincter muscle as affected by hypoxia. The ordinate shows the amplitude in grams of sphincter contractions above baseline (total isometric tension) caused by histamine. The abscissa is the molar concentration of histamine in the bath. Closed circles represent sphincter muscle responses to histamine in Krebs solution equilibrated with 95% O_2−5% CO_2. Open circles represent the responses of the same strips to histamine in Krebs solution equilibrated with 95% N_2−5% CO_2. Each point is the mean ± SE for 10 strips. Asterisks indicate a significant difference between the two values obtained for each concentration. The contractions produced by histamine in the absence of oxygen are significantly less than those produced by histamine in the presence of oxygen.

is, in fact, evidence to suggest that most of the maintained closure of that sphincter is attributable to this myogenic tone (22). Myogenic tone has also been demonstrated in the oblique muscle layer of the opossum stomach (5), a layer that is continuous with the muscle of the esophagogastric sphincter, and in the pyloric and ileocecal sphincters (4, 5).

Studies of myogenic tone in tibial artery muscle (18) revealed that it depends upon oxidative phosphorylation, while drug-induced contractions are little affected by inhibitors of oxidative phosphorylation. This suggests a difference between the energy source that supports myogenic tone and that supporting drug-induced contractions. Similar observations have been made in a variety of other smooth muscle preparations.

Myogenic tone in muscle from the lower esophageal sphincter seems to be unusual, also, in that it is resistant to calcium deprivation. It is not abolished by severe reduction of calcium concentration in extracellular fluid, nor by excess magnesium (23).

The experiments reported here support the idea that active isometric tension in muscle of the gastrointestinal sphincters and in gastric fundus, like that in certain other kinds of smooth muscle, is entirely aerobic. In contrast, neurogenic contractions of other parts of the esophagus and drug-induced contractions of the esophageal sphincter can be sustained, at least in part, anaerobically.

Since the isometric tone of sphincteric muscle seems to be almost totally aerobic, it seems reasonable to propose that tissue hypoxia could be a means for reduction of the level of tone in sphincters. Such tissue hypoxia could result from a systemic disorder or from compromised vascular perfusion of sphincteric regions. This could account, in part, for the frequency with which reflex esophagitis occurs in patients following thoracic or abdominal surgery, during which hypoxemia is a common occurrence.

The finding that the off-response of circular muscle and the duration response of the longitudinal muscle are quite resistant to hypoxia (as compared to sphincter tone) was surprising. The three kinds of contractions have previously been considered to be neurogenic or myogenic on the basis of their sensitivity to tetrodotoxin. The off-response and the duration response are sensitive to tetrodotoxin while sphincteric tone is not. Hence, the former have been considered to be neurogenic, while the latter has been called myogenic. The best conclusion is that the autonomic nerves responsible for the former are not so oxygen dependent as other kinds of nerve tissue.

This is not to say that these autonomic nerves can function anaerobically. Their function did decline with higher degrees of hypoxia, and the metabolic inhibitors did indeed cause virtual or complete abolition of responses. These inhibitors, however, may inhibit other enzyme systems than those involved in respiration. From these data, it can only be said that these nerves are relatively resistant to hypoxia.

The fact that the response to histamine was depressed to some degree in hypoxic conditions may represent the fact that, to some degree this drug acts by an aerobic mechanism, though not exclusively so. Carbachol and substance P appear to excite the muscle by anaerobic mechanisms.

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