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Calcium in Smooth Muscle from the Opossum Esophagus (40681)

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In the distal esophagus of the opossum, the longitudinal and circular layer of the esophageal body and the sphincter are smooth muscle. These muscles contain intrinsic nerves that can be stimulated electrically. Such stimulation by an electrical field leads to contraction in the body of the esophagus, whereas it leads to a relaxation of the high resting tension (or tone) in muscle from the sphincter. Neurogenic contractions in muscle strips from the body of the esophagus are more sensitive to reduction of calcium in the bathing medium than are both the tone and the neurogenic relaxation in strips of sphincter muscle (1). This suggests that the source of calcium which maintains tone in the lower esophageal sphincter is different from the one which allows contraction of muscle from the esophageal body. To investigate this possibility, we compared the content and efflux of calcium in transverse muscle strips cut from the lower esophageal sphincter to that in transverse muscle strips cut from the body of the opossum esophagus.

Methods. The esophagus with a cuff of stomach was removed from mature opossums under barbiturate anesthesia (2). The specimen was opened along the lesser curvature of the stomach and pinned flat on a wax block in a dissection bath. The bath was gassed with 95% O_2–5% CO_2 and its temperature was kept at 36°. The bathing solution contained 15 mmole/liter glucose and (in meq/liter): sodium 139, potassium 4.6, magnesium 2.2, chloride 125, bicarbonate 22, phosphate 3.5, and sulfate 2.3. The calcium concentration in the bathing solution was 2.5 meq/liter. This is about half the concentration generally used (1, 3). This change is based on our finding of this concentration of ionized calcium in the venous blood of five opossums.

Muscle strips, about 2 mm wide and 10 mm long, were cut from four segments of the smooth muscle part of the esophagus. Segment I is the segment of esophagus where the dark red of the proximal striated muscle has just given way to the pale pink of the distal smooth muscle. Segment IV is the segment that constitutes the lower esophageal sphincter. Segments II and III are equally spaced between Segments I and IV. According to a recent study, the thickness of the longitudinal muscle layer remains about the same throughout the entire esophagus. The circular muscle layer doubles in thickness at the lower esophageal sphincter, largely due to the intervention of strands of connective tissue (4). Strips were briefly attached to a force transducer and exposed to an electrical field. All strips from Segment IV showed the tone that characterizes the sphincter, and they relaxed during stimulation. All strips from the body Segments I, II, and III showed "off responses," a phasic contraction after termination of the stimulus that is characteristic of the circular smooth muscle of the opossum esophagus (1). The period beginning with removal of the esophagus and ending with this testing of function never exceeded 30 min.

For determination of tissue calcium content, strips were blotted, weighed, dried in a vacuum, and reweighed. This was followed by ashing at 475° and dissolving the ash in acid. Complete dissolution was obtained by adding 1 ml of concentrated HNO_3 and 0.5 ml of concentrated HCl and heating in a crucible.

Extraction of vacuum-dried muscle strips with 0.5 *N* nitric acid has been used by us previously for measuring tissue sodium and potassium (2). It has been used by others for determination of calcium in smooth muscle (5). However, in four preliminary experiments, we found that calcium content determined by HNO_3 extraction was on the average 20% lower than when ashed muscle was completely dissolved in HNO_3 and HCl. In five additional experiments, we also showed that calcium content was not different when the strip was processed immediately and when it was processed after a period of in-

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cubation in the bath. Calcium content was 2.7 ± 0.2 mmole/kg in strips processed immediately and 2.5 ± 0.2 mmole/kg 2 hr after incubation. All subsequent determinations were done after 2 hr of incubation. Calcium content was measured by atomic absorption spectrophotometry (Perkin–Elmer, Model 303, Norwalk, Conn.) after diluting the samples with distilled water and adding acidic lanthanum (final concentration = 0.1% lanthanum and 0.5% HCl). All glassware and crucibles were acid-rinsed. Duplicate assays were performed on all specimens. Tissue calcium content is expressed in millimoles per kilogram of wet muscle; dry muscle weight is on the average 20% of wet muscle weight.

In nine animals strips were incubated for at least 2 additional hr after labeling of the bath solution with ^{45}Ca. Strips were then removed, blotted, and weighed before beginning the efflux study. For determination of Ca^{2+} efflux, strips were pinned into the opening of a small-bore plastic tube, through which a steady stream of 95% CO_2–5% CO_2 was maintained. Strips were dipped for exactly 2 min into each of 15 vials containing 2 ml of unlabeled bathing solution gassed with 95% O_2–5% CO_2 and kept at 36°C.

At the end of the washout period, the strips were again blotted, weighed, and then dissolved by shaking them overnight in glass vials containing 3 ml of a tissue solubilizer (NCS, Amersham/Searle, Arlington Heights, Ill.). Radioactivity in the dissolved samples was determined by liquid scintillation counting after adding 15 ml of scintillant (ACS, Amersham/Searle, Arlington Heights, Ill.) and 0.5 ml of a mixture of EDTA in trichloracetic acid. The amount of ^{45}Ca removed during each 2-min period and the amount remaining at the end of the washout procedure are expressed as a percentage of the total calcium uptake by the tissue. All values are expressed as the mean (X) ±1 SEM. Differences between the four esophageal segments were considered significant when the P value from the paired t test was less than 0.05.

Results. Total tissue calcium content. The calcium content is uniform in the four esophageal segments. The total tissue calcium content in the four esophageal segments (in mmole/kg of wet muscle) is: 2.7 ± 0.1 in Segment I, 2.8 ± 0.3 in Segment II, 2.6 ± 0.3 in Segment III, and 2.6 ± 0.3 in Segment IV after 2 hr of incubation in the Krebs medium.

Washout of calcium-45. Efflux from all esophageal segments is rapid during the first three periods with a halftime of 5 min. Efflux is slow during the subsequent 12 periods with a halftime of 29 min. In all nine animals tested, efflux from sphincter muscle is slower than efflux from the body of the esophagus, and consequently a greater fraction of the total ^{45}Ca uptake remained in sphincter muscle than in muscle from the body of the esophagus. Figure 1 shows an example of an efflux curve from sphincter muscle and from a strip of more proximal muscle in one animal. The mean percentage efflux during all 15 periods is given in Table I.

There is no significant difference in weight of strips from the four esophageal segments (see Table I). Also, there is no statistical correlation between strip weight and fractional efflux during any period ($r < 0.5$; $P > 0.05$).

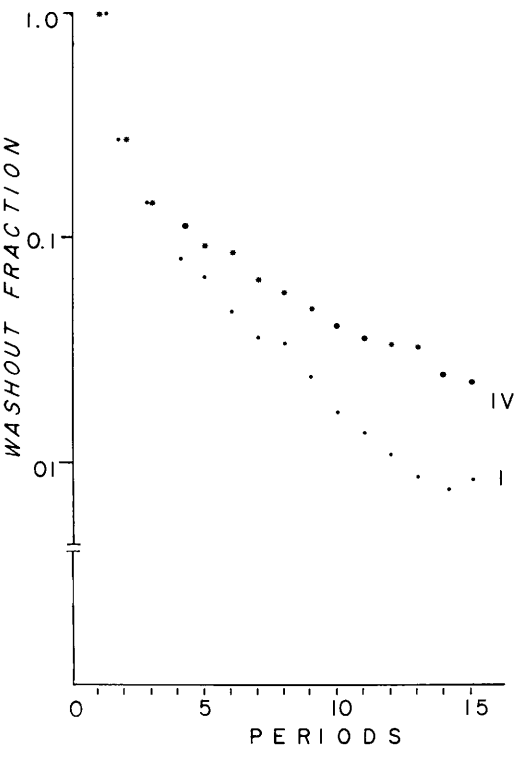

FIG. 1

TABLE I. MEAN PERCENTAGE EFFLUX FROM FOUR SEGMENTS OF ESOPHAGEAL SMOOTH MUSCLE (N = 9)

	Segment			
Period	I (Proximal) (133 ± 17 mg)	II (Middle) (127 ± 20 mg)	III (Distal) (141 ± 16 mg)	IV (LES) (156 ± 18 mg)
1	47.4 ± 3.4	47.0 ± 2.7	45.4 ± 3.5	40 ± 1.5[a]
2	12.4 ± 1.1	12.4 ± 0.6	13.1 ± 0.7	9.9 ± 0.3[a]
3	6.7 ± 0.7	6.8 ± 0.6	7.8 ± 0.7	6.0 ± 0.1
4	4.7 ± 0.4	4.8 ± 0.4	5.3 ± 0.2	4.4 ± 0.1
5	3.6 ± 0.2	3.8 ± 0.4	3.8 ± 0.4	3.5 ± 0.1
6	2.9 ± 0.3	2.7 ± 0.2	3.1 ± 0.3	3.2 ± 0.2
7	2.3 ± 0.2	2.3 ± 0.1	2.2 ± 0.2	2.4 ± 0.1
8	2.0 ± 0.1	2.2 ± 0.2	2.1 ± 0.3	2.1 ± 0.1
9	1.7 ± 0.1	1.9 ± 0.3	1.5 ± 0.3	1.9 ± 0.1
10	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.3	1.5 ± 0.1
11	1.3 ± 0.1	1.4 ± 0.2	1.3 ± 0.2	1.5 ± 0.1
12	1.2 ± 0.1	1.1 ± 0.1	1.0 ± 0.2	1.4 ± 0.01
13	1.2 ± 0.3	1.1 ± 0.1	1.0 ± 0.1	1.3 ± 0.1
14	1.0 ± 0.2	0.8 ± 0.1	0.8 ± 0.1	1.2 ± 0.1
15	0.8 ± 0.1	0.3 ± 0.1	0.8 ± 0.1	1.0 ± 0.1
Tissue	9.7 ± 1.3	9.7 ± 0.9	9.5 ± 2.0	18.5 ± 1.4[a]

[a] Refers to values where sphincter strips differ significantly from body strips. There was no significant difference between any two segments of the body of the esophagus.

Discussion. Our findings indicate that the total amount of cellular calcium is similar in the tonic muscle from sphincter and in phasic muscle from the body of the opossum esophagus; but muscle of the latter type exchanges calcium more readily with the bathing solution.

In the opossum, the tissue potassium content of sphincter muscle is substantially lower than that of muscle from the esophageal body. This is due to a lower cellular potassium concentration in sphincter muscle. In the cat, a gradient of tissue calcium content has been described in small bowel muscle (5). In contrast, our findings indicate that tissue calcium content in the smooth muscle segment of the opossum esophagus does not depend on location. Because of the high contribution of extracellular calcium to the total tissue calcium content, it is, however, possible that small differences in cellular calcium content are missed by our method (2, 3). Different methods such as the ones using La^{3+} or $^{40}Ca^{2+}$–$^{45}Ca^{2+}$ (6) could therefore still demonstrate differences of cellular calcium content between esophageal body and sphincter muscle.

Sphincter muscle exchanges calcium with the bathing solution less rapidly than muscle from the body of the esophagus so that the amount of ^{45}Ca remaining in sphincter muscle is substantially greater than that in body muscle after a prolonged washout. This finding is consistent with our previous observation that muscle from the sphincter shows less functional impairment on reduction of calcium in the bathing solution than muscle from the body of the esophagus. Thus, contractions in the body of the esophagus were effectively abolished 15 min after calcium removal, whereas the tone in the sphincter was only reduced (1).

There could be less efficient exchange of the calcium in sphincter muscle than in muscle from the body of the esophagus because the greater thickness of the sphincter (4) retards diffusion. Thus, extracellular calcium deep in the tissue could account both for the ability of sphincter muscle to maintain its mechanical performance after removal of calcium from the bathing medium and for the greater amount of calcium remaining in the sphincter after washout. There are, however, several arguments against explaining our findings on this basis. The mechanical performance of the sphincter differs from that of the body also in respect to sensitivity to magnesium, strontium, and nitroprusside, all indicating a different action of calcium in sphincter from that in body (1).

A difference in accessibility of the extracellular space also seems unlikely, since all strips are cut so that they were never larger than 2 mm, which is about the maximal wall

thickness. Our experience with markers of extracellular space indicates furthermore that exchange of extracellular fluid is more than 90% complete in muscle strips of this size within about 20 min (2). Furthermore, the sphincter strips were actually lighter than body strips in several animals, but initial washout of calcium in these animals was still less and the fraction remaining in the tissue was still larger than in strips from the esophageal body. Lastly, there is no gradient of decreasing washout from segments I to III, even though there tends to be an increase of strip weight due to greater muscle thickness. Accordingly, there is for none of the washout periods a correlation between strip weight and washout rate.

It is surprising that the higher tissue calcium remaining in the sphincter at the end of the 15 washout periods is largely due to slower removal during the first washout periods, the periods which reflect washout from the extracellular rather than the intracellular space. One explanation is that calcium in sphincter muscle is partially bound to extracellular structures. Such extracellular calcium binding has been shown to change the appearance of cellular calcium efflux in taenia coli, for example (7). Another explanation is that early cellular washout rather than extracellular washout accounts for the observed differences. It has been found by some that calcium washout from gastrointestinal smooth muscle can be separated into two early and one late phase. The second phase with a halftime between 4 and 7 min accounted for much of the washout between 2 and 10 min. It was assumed to be due to early cellular washout, whereas late cellular washout had a halftime of between 22 and 29 min and accounted for only a small fraction of total washout (8).

So far two main differences between phasic and tonic types of gastrointestinal smooth muscle have been described with regard to calcium storage and activation systems. First, tonic smooth muscle contains a relatively large smooth endoplasmic reticulum, which is believed to be an important store for intracellular calcium (9). Secondly, verapamil preferentially blocks phasic contractions of muscle from the gastric antrum, whereas nitroprusside blocks preferentially the tonic contraction of the gastric fundus indicating differences in calcium activation systems (10).

The washout of K^+, Na^+, and Cl^- is different in tonic as compared to phasic vascular smooth muscle (11). Our present findings provide preliminary evidence that there are differences in calcium handling between tonic and phasic types of esophageal smooth muscle as well. The above-mentioned structural differences in the tunica muscularis of the esophageal body and the lower esophageal sphincter (4), however, raise the possibility that these variations in calcium handling result from differences in the arrangement of smooth muscle cells rather than from differences in their individual properties.

Summary. Transverse muscle strips were cut from four segments of the opossum esophagus. Segment I was the segment of smooth muscle bordering on the striated muscle proximally and Segment IV was the distal segment which includes the lower esophageal sphincter. Segments II and III were the two central fourths of the smooth muscle esophagus. The tissue calcium content was determined in strips coming from the four segments after ashing and extraction of strips in concentrated HCl–HNO_3. The calcium content (in mmole/kg wet muscle ± 1 SE) was 2.70 ± 0.1 in Segment I, 2.8 ± 0.3 in Segment II, 2.6 ± 0.3 in Segment III, and 2.6 ± 0.3 in Segment IV after 2 hr of incubation.

After incubation of strips coming from the four segments, efflux of ^{45}Ca was measured during 15 two-min periods. Efflux was rapid at first with a halftime of 5 min and slowed after three periods to a halftime of 29 min. In all nine animals tested, efflux from the lower esophageal sphincter was slower than that from muscle of the esophageal body. Thus, calcium content in the smooth muscle esophagus does not depend on location. However, tonic esophageal smooth muscle (sphincter) exchanges calcium less rapidly with the bathing medium than the phasic-type smooth muscle from the body of the opossum esophagus.

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