A SHIFT OF CELLULAR CALCIUM TO A MORE SLOWLY EXCHANGEABLE FRACTION DURING CONTRACTION IN GUINEA PIG TAENIA COLI

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It is now generally agreed that Ca ion is essential for the contraction of smooth muscle. On the role of Ca ion in the contraction cycle of smooth muscle, many electrophysiological studies have suggested that the action potential of the muscle cell is associated with an inward movement of Ca ions from anionic sites of cell membrane or from extracellular space and this Ca flow may initiate contractile response (1, 2). It has also been suggested from radioisotope studies (3-6) that contraction in smooth muscle may be triggered and maintained by release of cellular Ca and/or by influx of extracellular Ca.

Although Ca ions activating contractile protein of smooth muscle are said to be inactivated and stored in the cell until expelled (7), the mechanism is still obscure. In previous experiments, tissue Ca of taenia coli was tentatively divided into two fractions; a fraction which exchanges within 4 min and another fraction which is exchangeable but does not exchange within 4 min, the latter more slowly exchangeable fraction was named as 'tightly bound fraction'(TBF) (4). The size of TBF increased during tonic contraction induced by carbachol, pilocarpine (3), histamine (4), barium (5) and 40 mM potassium (5, 6), and decreased during abolition of tension by specific antagonists of the stimulants (3, 4), metabolic inhibiting factors and factors inhibiting active Na transport (8, 9). From these data, it was suggested that the increase in the size of TBF, i.e., a shift of cellular Ca to a more slowly exchangeable fraction, could be related to contraction of smooth muscle, and the movement of Ca related to metabolism.

In the experiment reported here, the effect of external phosphate on the slowly exchanging Ca fraction, the change in the fraction in the course of muscle contraction and relaxation and exchange kinetics of the fraction were studied to clarify the nature of the shift of cellular Ca to a more slowly exchanging fraction in smooth muscle of taenia coli and the role of this Ca movement in excitation-contraction coupling in smooth muscle.

METHODS

Strips of taenia coli were isolated from male Hartley strain guinea pigs weighing about 500 g. Tyrode solution containing NaCl 136.8, KCl 2.7, CaCl₂ 2.5, MgCl₂ 1.0, NaH₂PO₄ 0.4, NaHCO₃ 11.9 and glucose 5.5 mM was bubbled with 95% O₂ and 5% CO₂ mixture

at 37°C. Tension changes were recorded isometrically. Tissue Ca and extracellular ^{14}C -sorbitol space were determined as described previously (5, 8).

For ^{45}Ca efflux experiments, muscle strips were allowed to take up ^{45}Ca for at least 30 min in Tyrode solution without or with hypertonically added 40 mM K or 5.4×10^{-6} M histamine. Previous studies have shown that most of the tissue Ca in taenia coli was exchangeable and exchanged within 15 to 30 min (4, 5, 10). The more slowly exchanging fraction also increased 15 to 30 min after addition of the stimulants (4-6). After incubation, the muscle strips were washed with nonradioactive Tyrode solution, this being the same chemical composition as the incubation medium. In the first series of experiments the strips were removed from the bath after 2, 4, 6, 8 or 15 min wash period and radioactivity remaining in the strips was counted. The resultant "desaturation curve" was plotted on a uniform scale as mEq Ca/Kg wet tissue vs time after the start of wash (Fig. 3-A.). TBF corresponds to the value obtained after 4 min wash period in this experiment. In the next series of experiments the muscle strips were washed every min and the radioactivity in the strips during the efflux period was calculated from radioactivity in each effluent and that remaining in the strips after a 15 min wash period. The "efflux curve" obtained from this experiment was plotted on a semilogarithmic scale (Fig. 3-B). There was a slight difference in the curves showing initial faster exchange in the "desaturation curve" and the "efflux curve" which may be due to the fact that radioactive solution adhering to muscle strips was carried with the strips into wash solution (11), and the curve showing initial faster exchange was not shown in the "efflux curve" to avoid the uncertainty.

RESULTS

1. Effect of external phosphate on the size of TBF

When the external phosphate concentration was lowered from control level of 0.4 mM to 0 mM, the size of TBF decreased from 1.70 ± 0.09 mEq/Kg wet tissue (7) to 0.95 ± 0.22 mEq/Kg (8) although tissue Ca did not change significantly (Fig. 1). Similar results have been reported by Schatzmann (12) and Goodford (13). In the presence of 40 mM K, muscle strips contracted and both tissue Ca and TBF increased as reported previously (5, 6). Increments in the size in the presence of 40 mM K were scarcely affected by change in the external phosphate concentration (Fig. 1). Tension development by 40 mM K was also shown to be unaffected by external phosphate concentration (14).

A part of TBF which increased during contraction is termed as "accumulated Ca" for distinction, and the present results are summarized as; the "accumulated Ca" were not affected by the change in external phosphate concentration although a part of slowly exchangeable fraction was directly affected by the same factor.

2. Change in "accumulated Ca" in the course of muscle contraction and relaxation

The addition of 40 mM K to Tyrode solution bathing taenia coli resulted in a prompt rise of tension followed by a steady level which was maintained as long as the K concentration was elevated. During the tonic contraction "accumulated Ca" increased gradually. Tissue Ca also gradually increased during the period. When 40 mM K was washed out,

FIG. 1. Effect of phosphate concentration in the medium on tissue Ca and TBF in guinea pig taenia coli.

Tissue Ca (squares) was measured by atomic absorption spectrophotometer. TBF (circles) was determined as a Ca fraction which did not exchange within 4 min. Value in the absence (open symbols) and in the presence of hypertonically added 40 mM K (filled symbols) are shown. The difference between TBF in control tissue and that in K-treated one, named as "accumulated Ca", was not affected significantly by the change in phosphate concentration in the medium (shaded area). Standard error of mean is shown by vertical line.

FIG. 2. Changes in tension, "accumulated Ca" and tissue Ca in the course of muscle contraction and relaxation in guinea pig taenia coil.

A: Tension change recorded isometrically.

B: Change in "accumulated Ca" calculated from Table 1 as a difference between control TBF and that treated with 40 mM K or 5.4×10^{-6} M histamine.

C: Change in tissue Ca calculated from Table 1 similar to "accumulated Ca".

TABLE 1. Changes in tissue Ca and TBF in the course of muscle contraction and relaxation.

Condition	Time (min)	Ca (mEq/kg wet tissue) (mean $\pm$ S.E.)				Histamine	
		Control		40 mM K		Tissue Ca	TBF
Stimulant added	0	Tissue Ca	TBF	Tissue Ca	TBF		
	5	4.36 $\pm$ 0.15 (10)	1.45 $\pm$ 0.10 (8)				
	10	4.40 $\pm$ 0.23 (8)		4.47 $\pm$ 0.10 (8)	1.54 $\pm$ 0.09 (7)		
	15	4.40 $\pm$ 0.16 (8)		4.75 $\pm$ 0.15 (8)	1.76 $\pm$ 0.22 (8)		
	19			5.09 $\pm$ 0.11 (6)	2.31 $\pm$ 0.24 (8)	4.30 $\pm$ 0.15 (7)	
Stimulant removed	30	4.40 $\pm$ 0.11 (10)	1.30 $\pm$ 0.10 (8)	5.55 $\pm$ 0.19 (8)	2.40 $\pm$ 0.09 (8)		2.33 $\pm$ 0.12 (7)
	32		1.50 $\pm$ 0.07 (8)		2.85 $\pm$ 0.07 (8)	4.25 $\pm$ 0.22 (7)	2.88 $\pm$ 0.10 (8)
	34				2.49 $\pm$ 0.10 (8)		
	35				2.57 $\pm$ 0.09 (8)		2.63 $\pm$ 0.18 (8)
	39			5.10 $\pm$ 0.35 (8)			1.98 $\pm$ 0.21 (8)
	40			4.73 $\pm$ 0.36 (8)	2.10 $\pm$ 0.09 (8)		
	45	4.37 $\pm$ 0.20 (7)	1.50 $\pm$ 0.10 (8)	4.39 $\pm$ 0.20 (8)	1.61 $\pm$ 0.08 (8)	4.32 $\pm$ 0.18 (7)	1.66 $\pm$ 0.15 (7)

40 mM K or 5.4×10^{-6} M histamine was added at 0 min and removed at 30 min.

TBF is a Ca fraction which is exchangeable but did not exchange within 4 min.

Numbers in parenthesis represent number of experiments.

tension rapidly returned to the control level. However, approx. 15 min were required for both "accumulated Ca" and tissue Ca to return to control levels. Similar results on "accumulated Ca" and tension were obtained using 5.4×10^{-6} M histamine although tissue Ca remained unchanged in this case. These results are shown in Table 1 and the changes in tension, "accumulated Ca" and tissue Ca are illustrated in Fig. 2.

Shimo and Holland (15) reported that the membrane depolarization induced by 40 mM K returned to original level within few min after 40 mM K had been removed. The "accumulated Ca", however, remained still high at this time (Fig. 2). In the next experiments, a concentration of K in the medium was elevated to 40 mM for 30 min, then was lowered to control level. Two to three min later, 5.4×10^{-6} M histamine or 40 mM K was added. Tension development by 40 mM K did not change but histamine induced only a small tension after the K-treatment.

These results show that Ca ions gradually accumulate in slowly exchanging fraction during contraction of the smooth muscle and slowly disperse after muscle relaxation. It is also suggested that "accumulated Ca" may not be a source of Ca ions for muscle contraction but a depot for Ca which has been utilized for muscle contraction.

3. Exchange kinetics of "accumulated Ca"

In taenia coli the exchange kinetics of Ca cannot be fitted to a unique sum of first order processes (5, 11), hence the division of tissue Ca into fractions on this basis is unreliable. An attempted analysis of ^{45}Ca influx and efflux curves showed some apparent phases of Ca exchange in taenia coli (10, 11, 16, 17) although the phases may not invariably coincide with cellular Ca components (2, 5, 11). In the following experiments an attempt was made to find the exchange kinetics of "accumulated Ca" from ^{45}Ca efflux experiments. The results are expressed in "desaturation curve" (Fig. 3-A) and "efflux curve" (Fig. 3-B) (see Methods). As can be seen in the figures almost 95% of tissue Ca exchanged within 15 min and the tentative analysis of "efflux curve" revealed that most of the tissue Ca exchanged following three phases; initial rapidly exchanging component, the second component with a half-time of approx. 2 min and the third component with a half-time of approx. 7

FIG. 3. Effect of 40 mM K and 5.4×10^{-6} M histamine on ^{45}Ca "desaturation curve" (upper) and ^{45}Ca "efflux curve" (lower) guinea pig taenia coli.

Tissues were loaded in labeled Tyrode solution without or with 40 mM K or histamine for at least 30 min. Thereafter efflux went on in nonradioactive Tyrode solution of the same chemical composition as the incubation medium.

For further details, see text.

min. When 5.4×10^{-6} M histamine was added to both ^{45}Ca incubation and wash solution, ^{45}Ca efflux was clearly slowed. The "desaturation curve" showed that significant differences between histamine-treated and those which served as control existed at the 4th and 6th min of efflux. The "efflux curve" also showed that the difference was mainly due to the decrease in the size of the second component and the increase in that of the third. In the presence of 40 mM K, ^{45}Ca uptake increased and the most of the extra ^{45}Ca in 40 mM K-treated tissue was attributed to the increase in the third component.

These data suggest that a part of cellular Ca, which exchanges with a half-time of approx. 7 min, increases during contraction of taenia coli.

DISCUSSION

The present experiments showed that a cellular Ca which exchanged with a half-time of approx. 7 min increased during tonic contraction in taenia coli and gradually decreased after muscle relaxation.

In longitudinal muscle strips of guinea pig small intestine Lüllmann and Siegfriedt (18, 19) reported that a Ca fraction with a half-time of 4 min decreased and that with a half-time of 22 min increased during contraction induced by 40 mM K. They suggested that the change in the relative size of the two cellular fractions in the presence of high potassium concentration is a result of a transfer of Ca from a loosely bound fraction to a more firmly bound one. This data is compatible with the present one which exhibited a shift of Ca between the two fractions during contraction. In these experiments stimulants were added during both ^{45}Ca loading and efflux periods or only during the latter one. A similar result was obtained when the stimulants were added only in the ^{45}Ca loading period. Van Breemen and Daniel (20) demonstrated a shift of Ca to a more slowly exchangeable fraction during contraction in the rat uterus by a slower efflux of ^{45}Ca from uteri which were exposed to 191 mM K or acetylcholine during loading with ^{45}Ca than from the control uteri which were not stimulated during loading. The contraction of intestinal muscle by electrical stimulation (21) and of uterine muscle by 44 mM K (22) during ^{45}Ca loading period also increased a residue of ^{45}Ca in the muscle after a wash period of the isotope. From these and the present data, it can be said that slowly exchanging Ca ("accumulated Ca") increases during contraction in taenia coli and also in other smooth muscle tissues.

Krejić and Daniel (22) suggested that contraction delayed ^{45}Ca fluxes due to the sequences of the mechanical events accompanying the contraction, e.g., changes in diffusion path through the extracellular space in rat uterine tissue. In taenia coli, however, ^{45}Ca fluxes were not slowed or even increased during contraction (3, 5, 11, 23), suggesting that the slowed ^{45}Ca movement observed in the present data is not the result of slowed diffusion of ^{45}Ca in the extracellular space.

The Ca accumulation during contraction in taenia coli has been postulated to be an active process depending on an aerobic carbohydrate breakdown and also related to an active Na transport (8, 9). Active Ca accumulation into some cellular fraction is necessary in order to maintain the ionic Ca concentration around contractile filaments at low level

(2, 7, 20). In skeletal muscle the endoplasmic reticulum is responsible for the active accumulation of Ca from the sarcoplasm to maintain muscle relaxation (24), however, the sarcotubular system is poorly developed in smooth muscle when compared to the elaborately organized structure found in skeletal and cardiac muscles (25). As for the Ca accumulating site in smooth muscle, Totsuka (26) demonstrated the ATP extrasplitting, ^{45}Ca binding and relaxing activities of microsomal fraction prepared from dog small intestine. Further, Kato et al. (27) showed electron microscopically that Ca distributed in myofilament and matrix of mitochondria during contraction, and around pinocytotic vesicle and in cell interstitium during relaxation of uterine muscle.

Goodford (7) has proposed that any Ca entering the smooth muscle cell is rapidly removed, so long as sufficient metabolic energy is available, from the cytoplasm into storage site where it forms a microcrystalline precipitate of Ca phosphate which dissolves and liberates Ca slowly into the extracellular bathing solution and that the microcrystalline deposit may even be present in the absence of phosphate in the medium. In the present data a part of slowly exchanging Ca fraction but not the "accumulated Ca" decreased when the external phosphate concentration was lowered. The "accumulated Ca" exchanged with a half-time of approx. 7 min during contraction (Fig. 3-B), which does not contradict the fact that the "accumulated" Ca decreased to approx. 10% within 15 min after muscle relaxation (Fig. 2). These observations suggest that the release of "accumulated Ca" might be unaffected by the changes associated with muscle contraction or relaxation. The data seem to support the proposal of Goodford (7) that Ca accumulation is an active process while its release is inactive.

From the data, it is proposed that cytoplasmic Ca ion is continuously supplied by Ca influx and/or release of loosely bound membrane Ca during tonic contraction in taenia coli. The Ca ion which acted on contractile protein is actively and continuously accumulated in a slowly exchangeable Ca fraction. The accumulated Ca disperses slowly into the extracellular solution. There is no evidence to suggest that the accumulated Ca is released again into cytoplasm to induce contraction.

SUMMARY

A shift of cellular Ca to a more slowly exchangeable fraction has been found during tonic contraction in guinea pig taenia coli. A part of the Ca fraction which increased during contraction was termed as "accumulated Ca" and the nature of the Ca fraction was investigated.

1. Lowering the phosphate concentration in the external medium from control level of 0.4 mM to 0 mM decreased a part of the slowly exchangeable fraction but not the "accumulated Ca".

2. The addition of 40 mM K or 5.4×10^{-6} M histamine resulted in a prompt rise of tension followed by a new steady level which was maintained as long as the stimulant existed. During the tonic contraction the "accumulated Ca" increased gradually. When the stimulants were removed, muscle tension returned rapidly to the original level while

almost 15 min was required for the "accumulated Ca" to attain the control level. It is suggested that the "accumulated Ca" is not a source of Ca ion for muscle contraction but a depot of Ca which has been utilized for contraction.

3. From ^{45}Ca efflux experiments, the "accumulated Ca" was shown to exchange with a half-time of approx. 7 min.

4. From the data, it is proposed that the Ca ion acting on contractile protein is actively and continuously accumulated in a slowly exchangeable Ca fraction during tonic contraction in taenia coli.

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