MOVEMENTS OF CALCIUM DURING TENSION DEVELOPMENT INDUCED BY BARIUM AND HIGH-POTASSIUM IN GUINEA PIG TAENIA COLI

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In previous publications it was reported that barium in concentrations from 0.5 to 20 mM induced tension development in isolated guinea pig taenia coli, which response was dependent on the presence of external calcium (1), and that total Ca content did not change but ^{45}Ca uptake of the cellular fraction which did not exchange within 4 minutes increased above control level during Ba-induced tension development (2). It was also mentioned that Ba increased Ca entry in taenia coli and that this Ca probably plays an essential role during tension development (2).

On the other hand, hypertonically added 40 mM K-induced tonic tension development in taenia coli was noted to be composed of phasic and tonic phases and it was proposed that in the phasic contraction, sufficient Ca is released from a cellular site to initiate contraction, whereas in the tonic contraction enough Ca crosses the membrane to initiate and also to maintain contraction(3). However, the movement of Ca in the above processes has not been studied systematically. This work was undertaken to study the movement of Ca in taenia coli during Ba- and high-K-induced tension developments by investigating the total Ca, ^{45}Ca uptake and efflux and ^{45}Ca uptake of the cellular fraction which did not exchange within 4 minutes.

A part of this work has already been reported (2, 4).

METHODS

Strips of taenia coli isolated from white male guinea pigs were suspended in Tyrode solution of the following composition (mM); NaCl 136.8, KCl 2.7, CaCl_2 2.5, MgCl_2 1.0, NaH_2PO_4 0.4, NaHCO_3 11.9 and glucose 5.5, at 37°C, and gassed with 95% O_2 and 5% CO_2 mixture. Ba and high-K Tyrode solutions were made hypertonically by adding BaCl_2 or KCl crystals to the medium to give final concentrations of 0.5 and 20 mM Ba and 40 mM K, respectively. Tension changes were recorded isometrically by mechano-electrotransducer.

^{45}Ca uptake: In the first series of experiments, muscles equilibrated for one hour in normal Tyrode solution were incubated in a medium containing 0.5 mM Ba or 40 mM K

and ^{45}Ca . In the next series, the equilibrated muscles were soaked in a medium with 0.5 mM Ba or 40 mM K for 15 minutes and then ^{45}Ca was added to the medium for incubation. Activity of ^{45}Ca in the medium was 1×10^6 counts/min. ml. After each specified incubation period, the strips were drawn across a sheet of filter paper (Toyo Roshi Kaisha, Ltd. No. 4) to remove adhering solution, weighed, ashed and radioactivity was counted using proportional gas flow counter in the same manner as described in another paper (4).

^{45}Ca uptake (4 min wash): The experiment was similar to that in the preceding paragraph except that the strips were washed three times in 4 minutes at the end of each incubation period with a non-radioactive Tyrode solution of the same chemical composition as the incubation medium. This procedure was the same as that in former reports (2-4).

Tissue Ca: Strips were treated in the same manner as in isotope experiments and the Ca content was determined using atomic absorption spectrophotometer (4). Results were expressed as mEq/kg wet tissue uncorrected for extracellular space.

^{45}Ca efflux: A strip weighing about 10 mg was fastened to a glass holder with nylon gut and after a 10 minute equilibration period in Tyrode solution, 0.6 g tension was applied to the muscle. The muscle was then transferred to a test tube containing 2 ml of radioactive solution (200 μCi ^{45}Ca /ml) of the same composition as the inactive solution and loaded with ^{45}Ca for three hours. A series of test tubes containing 2 ml of inactive Tyrode solution oxygenated through a thin polyethylene tube was prepared, however, the first and the second tubes contained 5 ml inactive solution in order to wash away the radioactivity which had contaminated the glass holder during the loading period. After loading, the muscle was washed in the first tube for one minute and then washed successively in each tube for 10 minutes. Aliquots of 0.5 ml of the solution in each tube was evaporated in a planchette and radioactivity was counted in a proportional gas flow counter. The radioactivity remaining in the muscle was also counted as described above.

^{45}Ca was supplied by International Chemical and Nuclear Corp., U.S.A., as $^{45}\text{CaCl}_2$.

RESULTS

I. Tension changes

The typical tension change curves induced by the addition of 0.5 and 20 mM Ba and 40 mM K are shown in Fig. 1. Although both concentrations of Ba induced tension development, the one induced by 0.5 mM Ba became irregular and that induced by 20 mM Ba gradually decreased with time. These results are the same as in a former report (1).

II. Tissue Ca

Ca content of the muscle in normal Tyrode solution was about 4.4 mEq/kg wet

FIG. 1. Tension change curves induced by 0.5 and 20 mM Ba and 40 mM K in isolated guinea pig taenia coli.

FIG. 2. Changes in tissue Ca and ^{45}Ca uptake of taenia coli in the presence of 0.5 mM Ba(a) and hypertonic 40 mM K(b).

Left ordinate, ^{45}Ca content of the tissue in $\text{cpm/kg wet tissue} \times 10^{-6}$. Right ordinate, tissue Ca in mEq/kg wet tissue . These scales were selected so that points representing total and radioactive Ca would coincide when there has been complete exchange. ^{45}Ca and Ba or high-K were added at time 0 following the one hour equilibration in normal Tyrode solution. Each point is an average of 8 to 15 determinations and $\pm$ S.E. indicated where possible.

■, ● : Total and radioactive Ca in normal Tyrode solution respectively (a and b).
□, ○ : Total and radioactive Ca in the presence of Ba(a) and 40 mM K(b), respectively.

FIG. 3. Changes in tissue Ca and ^{45}Ca uptake of taenia coli in the presence of 0.5 mM Ba(a) and hypertonic 40 mM K(b).

Ba or high-K was added at time 0 and 15 minutes later, ^{45}Ca was added. Other details as in Fig. 2.

tissue throughout the period of observation. It is the lowest value reported (5–8). As shown in Fig. 2-a, 0.5 mM Ba failed to alter the tissue Ca. Similar results were obtained with 20 mM Ba. In the presence of 40 mM K, tissue Ca did not differ from control values during the first 5 minutes but after 10 minutes there was a gradual and steady rise for up to 30 minutes (Fig. 2-b).

III. ^{45}Ca uptake

Fig. 2 shows the results of experiments in which ^{45}Ca uptake was measured. Changes in tissue Ca are also shown so that points representing total and radioactive Ca would coincide when there has been a complete exchange. The uptake of ^{45}Ca of the muscle in the normal Tyrode solution was almost complete in the first 10 minutes. In the case of 0.5 mM Ba, ^{45}Ca uptake significantly increased at 5 minutes when ^{45}Ca uptake of the muscle was almost complete (Fig. 2-a). In the case of 40 mM K, ^{45}Ca uptake did not differ from control values at 2 and 5 minutes but it became gradually higher than control and was almost complete 10 minutes after the addition of high-K, which is compatible with the change in tissue Ca (Fig. 2-b).

In the next series of experiments ^{45}Ca was added 15 minutes after the addition of Ba or high-K, when the muscle was thought to be equilibrated with the new solution, to test whether the changes in ^{45}Ca uptake seen at the initial stage of tension developments were the same 15 minutes later. Results are shown in Fig. 3. When ^{45}Ca was added 15 minutes after the addition of Ba, ^{45}Ca uptake only at 2nd and 5th minutes after the addition of ^{45}Ca was significantly lower than control values (Fig. 3-a) but that in 40 mM K medium was significantly higher than control values after 5 minutes following the addition of ^{45}Ca (Fig. 3-b).

IV. ^{45}Ca efflux

The typical efflux curve of ^{45}Ca in the normal medium for the period up to 220 minutes is shown in Fig. 4. The rate of ^{45}Ca efflux (count/min²) was calculated from the radioactivity in each test tube and plotted as a fraction of the initial rate. The amount of radioactivity in the muscle (count/min) was also calculated and plotted as a fraction of the amount at the beginning of the experiment. As shown in the figure, the decline of both lines was almost linear after 120 to 220 minutes but they did not become parallel during the period of observation and the average half time of the effluent counts and the muscle counts between 140 and 220 minutes were 73 and 110 minutes, respectively.

FIG. 4. Efflux of ^{45}Ca from taenia coli into normal Tyrode solution.

Abscissa, time after the beginning of wash out the taenia coli which had been loaded with ^{45}Ca for 180 minutes. Logarithmic ordinate scale along which both curves have been shifted so that they start from a common origin.

○ : The rate of ^{45}Ca efflux (counts/min²) as a fraction of initial rate.

● : The amount of radioactivity in the muscle (counts/min) at each time of observation, as a fraction of the amount at the start of the wash out.

FIG. 5. Effect of Ba(a) and hypertonic 40 mM K(b) on the efflux of ^{45}Ca from taenia coli.

Upper curve: Amount of radioactivity in the muscle.

Lower curve: Rate of ^{45}Ca efflux.

0.5 or 20 mM Ba(a) or 40 mM K(b) was added to the washing medium between two vertical lines. Other details as in Fig. 4.

FIG. 6. Changes in tissue Ca and ^{45}Ca uptake (4 min wash) of taenia coli in the presence of 0.5 mM Ba(a) and hypertonic 40 mM K(b).

The experiment was similar to that in Fig. 2 except that the strips of taenia coli were washed three times in 4 minutes at the end of each incubation period with a non-radioactive Tyrode solution of the same chemical composition as the incubation medium.

■, ▲: Total and radioactive Ca in normal medium remaining after 4 minutes wash.

□, △: Total and radioactive Ca in the presence of Ba (a) and high-K(b) remaining after 4 minutes wash.

Other details as in Fig. 2.

In the presence of 0.5 and 20 mM Ba, rate of ^{45}Ca efflux decreased sharply and the curve showing the amount of ^{45}Ca in the muscle showed a breach in the decline (Fig. 5-a). On the other hand, 40 mM K had scarcely any effect on the latter curve although the rate of efflux was slightly affected (Fig. 5-b). As ^{45}Ca uptake increased but tissue Ca did not change 5 minutes after the addition of 0.5 mM Ba, ^{45}Ca efflux was expected to increase. However, the expected initial increase in the Ca outflow was not observed, probably because the time interval of the collection of effluent was too long to reflect the transient changes.

V. ^{45}Ca uptake (4 min wash)

This series of experiments was designed to determine the amount of ^{45}Ca remaining in the muscle after a 4 minutes wash following incubation (see Methods), in order to determine the amount of ^{45}Ca in the slowly exchanging fraction which does not exchange within 4 minutes. As seen in Fig. 6-a, and b, ^{45}Ca remaining in the muscle in the presence of 40 mM K or 0.5 mM Ba was greater than the control values. Similar results were obtained with 20 mM Ba.

DISCUSSION

The loss of ^{45}Ca from taenia coli was reported to be composed of three (8) or four different fractions (7). However, Goodford (9) noted that the loss of ^{45}Ca did not follow a single exponential course at any time and the present data is in agreement with this. As the cellular Ca could not be divided into fractions using the ^{45}Ca efflux curve, it was decided to divide it into two parts using the results of ^{45}Ca uptake (4 min wash) experiment, in which the incubated muscle strips were washed for 4 minutes with non-radioactive solution to wash out the radioactivity in the rapidly exchanging fraction of the tissue which exchanges within 4 minutes. Similar experiments were performed with differing washing periods (2-4, 7, 8, 10), and the fraction which remained after 4 minutes wash was suggested to be closely related to tension development of taenia coli in the previous reports (3, 4). In the control medium, ^{45}Ca uptake of the muscle after 30 minutes incubation was 8.8×10^8 cpm/kg wet tissue and that which remained after 4 minutes wash was 3.6×10^8 cpm/kg, that is, 40% of the activity remained after wash.

The fraction which did and did not exchange within 4 minutes are named as the "loosely" and the "tightly bound fraction", respectively. In the presence of 0.5 mM Ba, the "tightly bound fraction" increased to 172% of the control value, which corresponds to 69% of the total ^{45}Ca activity. Higher concentration of Ba (20 mM) showed a similar tendency. These results demonstrate that the relative size of the "loosely bound fraction" decreased and that of the "tightly bound fraction" increased during Ba induced tension development, which probably reflected a movement of Ca to a more firmly bound form, and a delayed ^{45}Ca uptake and efflux has to be expected.

As for the experiment with 40 mM K, the "tightly bound fraction" increased to 158% of the control which corresponds to 49% of the total activity. That is, both total tissue Ca and "tightly bound fraction" increased in their absolute size but the increase in the re-

lative size of the "tightly bound fraction" was greater than that of total tissue Ca.

From these data, the movements of Ca during Ba and high-K induced tension developments are summarized as follows.

Ba induced tension development: Soon after the addition of Ba (early phase), Ca influx and probably also efflux increases but tissue Ca does not change. Later (late phase), both Ca influx and efflux decrease below control level without affecting the tissue Ca and the transfer of Ca towards the "tightly bound fraction" of the tissue is also observed during the process.

Although it was earlier reported that Ba increased Ca entry in taenia coli (2), the movement of Ca was not so simple a process. Entry of Ca increased only during the "early phase" but during "late phase" all the data indicated a firmer binding of the cellular Ca. In the former report (1) we noted that Ba induced tension development was dependent on the existence of external Ca and it was resistant to the removal of external Ca for longer period than high-K induced tension. These data suggest that the process of firmly binding of Ca and/or the firmly bound cellular Ca is more closely related to the "late phase" of Ba induced tension development. On the other hand, the "early phase" seems to be the result of the increased Ca exchangeability. As this juncture a similarity in the "early (phasic) phase" of both Ba and high-K induced tension development has to be considered. Both Ba and high-K produce a phasic contraction which is independent of metabolism (1, 3, 10). This process is as a result of release of Ca from a cellular site in the case of high-K as shown in the present and other data (3), but in the case of Ba induced phasic contraction the possibility of the existence of two processes has to be reckoned with, 1) a movement of Ca within the cell as in the case of high-K in the absence of metabolic energy and 2) an increased Ca exchangeability when energy is available. It is also possible that both these processes exist simultaneously or separately depending on conditions.

In the previous report (1) we noted that the factors which inhibit metabolism (DNP, glucose removal and anoxia) and those that inhibit Na transport (ouabain and Li substitution) affected the tension changes induced by Ba and high-K differently. Although the earlier factors are discussed above, the latter eludes explanation based on the present data.

High-K induced tension development: During the 5 minutes after the addition of high-K (early phase), tissue Ca, Ca influx and efflux do not change from control level but in the "late phase" both tissue Ca and Ca influx increase above control level but Ca efflux does not alter, and the Ca taken up in the tissue moves towards the "tightly bound fraction".

Urakawa and Holland (3) reported that hypertonic 40 mM K-induced tension development in taenia coli was composed of phasic and tonic phases and suggested that in the phasic contraction, sufficient Ca is released from a cellular site to initiate contraction, whereas in the tonic contraction enough Ca crosses the membrane to initiate and also to maintain contraction. These data were supported in other reports (2-4, 10). In the present data, the difference in 5th minute values of ^{45}Ca uptake and tissue Ca shown in Fig. 2-b and Fig. 3-b was attributed to the existence of the "early phase" and it was shown that during this phase (or phasic contraction) Ca exchange did not differ from control level suggesting

that the initiation of contraction may be related to the movement of Ca within the cell, e.g. release of Ca from a cellular site. During "late phase" or tonic phase, Ca was taken up from external medium and kept in the cell suggesting that the initiation and maintenance of tension may be related to the transmembrane Ca transport especially towards the "tightly bound fraction" which is believed to be closely related to tension development of taenia coli.

Hypertonic high-K had scarcely any effect on the ^{45}Ca efflux in the present data which is consistent with the report by Chuijo and Holland (7), although slight acceleration (8) or slight transient slowing of it (9) has also been reported.

During the "late phase" of high-K induced tension development, an increase of ^{45}Ca uptake (without and with differing washing periods after incubation) has been reported (2, 4, 7, 8). Further, in the experiment with longitudinal muscle of guinea pig small intestine, Lüllmann and Siegfriedt (11) tentatively divided the ^{45}Ca efflux curve into three parts, the relative size of the third fraction of which increased when 40 mM K was added to the external medium.

On the other hand, Imai and Takeda (12) concluded that the phasic response and a start of the tonic response of taenia coli depends upon the extracellular Ca for their initiation (and also for maintenance of tension in the case of the latter) and that the rest of the tonic response draws on a store of "bound" Ca for its evolution. The difference between the data obtained by Imai and Takeda (12) and ours may be due to the difference in the external Na and/or K concentrations as described elsewhere (4).

Recently, Shibata *et al.* (13) showed that ^{45}Ca uptake of taenia coli significantly increased during the contraction produced by 40 mM K but not by 10 mM Ba. However, as their experimental planing of ^{45}Ca incubation was different from ours, their results could not be compared with ours.

In this report the extracellular space of the tissue was not determined. During contractions induced by hypertonic 40 mM K in taenia coli the extracellular space was reported to decrease slightly (7) or not to change (10) which suggests that the increase in the tissue Ca and ^{45}Ca uptake during high-K induced tension development would not be affected very much by the change in the extracellular space. Although there is no information on the effect of Ba on extracellular space of taenia coli at present, it is difficult to assume that the extracellular space would increase in the presence of Ba when muscles are in isometric contractile state, and under isometric conditions the change would be minimum. If, however, the decrease in extracellular space be significant, then the decrease in ^{45}Ca uptake observed during Ba induced "late phase" could be the result of a squeezing out of the extracellular ^{45}Ca . This does not counter our argument as the above discussion is based mainly on the results of ^{45}Ca uptake (4 min wash) and ^{45}Ca efflux experiments which are not very much affected by changes in extracellular space.

SUMMARY

1. Changes in the uptake and efflux of ^{45}Ca and total Ca content of smooth muscle of guinea pig taenia coli were measured during Ba and high-K induced tension develop-

ment.

2. ^{45}Ca uptake increased 5 minutes after the addition of 0.5 mM Ba but 17 to 20 minutes later, both uptake and efflux of ^{45}Ca decreased. Total Ca content of the tissue did not change during the process but ^{45}Ca in the tissue which remained after 4 minutes wash following incubation increased. Similar results were obtained with 20 mM Ba.

3. In the presence of hypertonic 40 mM K, ^{45}Ca uptake and tissue Ca did not change during the first 5 minutes but 10 to 30 minutes later, both ^{45}Ca uptake and tissue Ca increased. ^{45}Ca efflux scarcely altered throughout the process. ^{45}Ca in the tissue remained after 4 minutes wash following incubation increased.

4. It is proposed that the early phase of Ba induced tension development is the result of the increased Ca exchangeability although the participation of the process of Ca release from a cellular site can not be overlooked. The late phase is related to the process of firmly binding of Ca and/or to the Ca already firmly bound at a cellular site. On the other hand, the early (phasic) phase of high-K induced tension development is the result of the release of Ca from a cellular site and the late (tonic) phase is related to the transmembrane Ca transport especially towards the tightly bound fraction of the tissue.

5. In conclusion it was discussed that although the tension development by both Ba and high-K consisted two phases (early or phasic and late or tonic phases) and Ca was directly involved in both cases, the movement of Ca was distinctly different from each other in the case of the tonic contraction but were comparable in the case of the phasic phase.

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