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CALCIUM DEPENDENT INOSITOL TRISPHOSPHATE-INDUCED CALCIUM RELEASE IN THE GUINEA-PIG TAENIA CAECI

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[Summary] The properties of the Ca stores in the skinned fiber bundles of the guinea-pig taenia caeci were studied by a novel method to measure Ca released from the stores using a fluorescent Ca indicator, fura-2. It is found that the capacity of the Ca stores amounts some 170 μmoles/liter cell water. There are at least two Ca release mechanisms, Ca-induced Ca release (CICR) and Ca release induced by inositol-1,4,5-trisphosphate (IP_3). Only about a half of the Ca is released by the Ca-induced Ca release, while IP_3 releases most of the Ca. The rate of the IP_3-induced Ca release is found to be strongly enhanced by submicromolar concentrations of Ca^{2+}.

Smooth muscle cells have intracellular Ca stores which release Ca in an amount sufficient for full activation of contraction in response to chemical transmitters (1). It has been shown that IP_3 induces Ca release from the Ca stores of smooth muscle cells (2,3,4) as in many other cells (5), and that it is produced rapidly in response to agonist stimulation (6). However, whether or not IP_3 concentration in the vicinity of the Ca store becomes high enough following a stimulus still remains to be seen, and further studies on the properties of the Ca stores are required before the physiological signal which causes Ca release is elucidated.

The present investigation was carried out to study properties of the Ca stores in smooth muscle cell using skinned fiber bundle preparations. It has been shown among others that the rate of IP_3-induced Ca release is dependent on Ca^{2+} concentrations at about the resting level. The finding may be important for the understanding of intracellular Ca mobilization.

METHODS

Thin fibre bundles, 150-200 μm wide, were dissected from taenia caeci of the guinea-pig and chemically skinned by 50 μg saponin/ml for 30 min (7).

ABBREVIATIONS: EGTA, ethylene glycol bis(β-aminoethyl ether) *N,N'*-tetra-acetic acid; IP_3, Inositol-1,4,5-trisphosphate; CICR, Ca-induced Ca release.

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The fibre bundle tied to a fine metal wire was placed in a glass capillary (i.d. 400μm), which was connected to pumps so that the solution could be rapidly changed. The capillary was mounted on the stage of an epi-fluorescence microscope and the change in the fluorescence intensity of fura-2 (8) in and around the fiber bundle was monitored to assay the amount of Ca released from the preparation. Excitation wave length was 340 or 380 nm. The emission was passed through a band pass filter (center wavelength, 500 nm) and focused onto a photomultiplier. Fura-2 being the only Ca buffer at the assay, it was possible to measure *total* Ca released from the preparation. Calibration revealed a linear relation between the total Ca and the change in the fluorescence intensity up to about half the maximum change. Many solutions used were similar to those described previously (7), but 20 mM NaN_3 was always added, and pH was 7.0. Temperature 20-21° C. IP_3 was purchased from Sigma. Results obtained from a set of fiber bundles are shown in the following, but they have been confirmed in different fibers.

RESULTS AND DISCUSSION

Fig. 1 shows the change in the fluorescence intensity of fura-2 in response to various stimuli. The fibre bundle was first treated with 1μM Ca^{2+} for 180 s in the presence of MgATP (Ca loading), and then Ca, ATP and EGTA were successively removed. Upon application of an assay solution containing both 50 mM caffeine and 10μM IP_3 there was a large fluorescence intensity change (*a*). However, without the Ca loading, there was only a very small increase (*b*) which was due to a direct effect of caffeine on the fura-2 fluorescence. Therefore the difference (*a*-*b*) shown in *d*, represents Ca released from the skinned fiber bundle. When only either caffeine (*e*) or IP_3 (*f*) was administered, the Ca signal was about 40 or 90 %, respectively, of that of the simultaneous application[1]. Ca "loading" without ATP produced no response (*g*). These results demonstrate that the skinned fiber bundles have Ca stores which actively accumulate Ca and release it in response to caffeine or IP_3.

Caffeine seems less effective than IP_3. This is shown in a different manner as follows. If caffeine assay was repeated without intervening Ca loading, there was no Ca response for the second assay (*h*). However, IP_3 assay following caffeine assay produced a definite Ca response (*i*), while caffeine assay after IP_3 assay did not elicit any Ca response (not shown).

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[1]One of the explanations for the difference in Ca responses to IP_3 and caffeine+IP_3 (*f* vs. *d*) may be that Ca^{2+} released by caffeine enhanced IP_3-induced Ca release which is Ca^{2+} dependent as shown by this study.

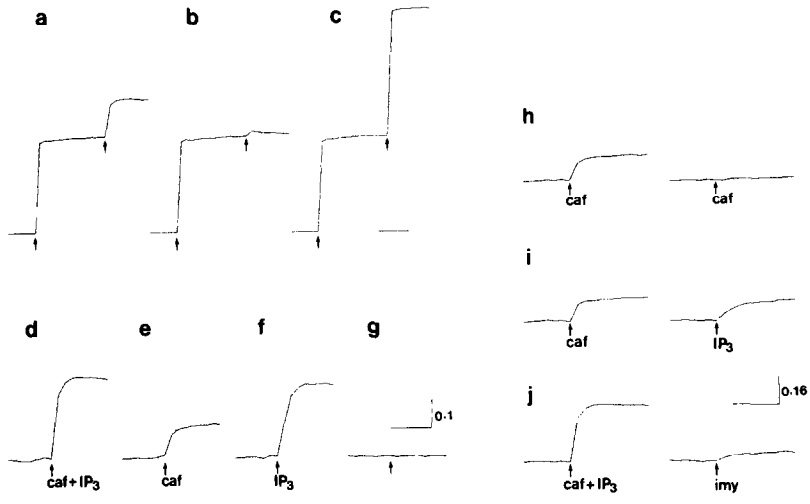

Fig. 1. Fluorescence intensity of fura-2 in the solution bathing a skinned fibre bundle of taenia. Excitation at 340 nm. *a*, The fibre bundle was incubated with Ca (pCa 6) in the presence of 4 mM MgATP for 180 s (Ca loading), then Ca and ATP was washed for 180 s until the first arrow when both EGTA and Mg were withdrawn and the shutter was opened. At the second arrow an assay solution containing 10 μM IP_3, 50 mM caffeine and 25 mM AMP was applied. Solution changes were carried out by a flush for 1 s to prime the dead space of the tubing followed by a slower continuous flow. But in the case of assay, only the flush was carried out. Fura-2 (43 μM) was introduced 60 s before the first arrow. *b*, Similar to *a* except that the Ca loading was omitted. *c*, Similar to *b*, but 100 μM Ca was added to the assay solution to obtain the maximum response. *d*, The difference (*a*–*b*) normalized with the maximum response. *e*, *f*, Similar to *d*, but only either caffeine* or IP_3 was present in the assay solution respectively. (*25 mM AMP always accompanied the caffeine assay solution.) *g*, Similar to *d*, but the Ca loading was carried out without ATP. *h*, Caffeine assay followed by another caffeine assay. *i*, Caffeine assay followed by IP_3 assay. *j*, Caffeine+IP_3 assay followed by 5 μM ionomycin assay. Horizontal calibrations, 30s. Vertical bars in *d*–*j*, fluorescence intensity change relative to the maximum change.

A Ca ionophore, ionomycin produced a Ca response comparable to that elicited by caffeine+IP_3 assay (not shown). Ionomycin assay immediately following caffeine+IP_3 assay produced very small response (*j*). Thus it is likely that the Ca stores observed have membrane boundary, and are probably the sarcoplasmic reticulums (1), and that most of the Ca in the stores can be released by the simultaneous application of caffeine and IP_3.

Fig. 2 shows the effect of pCa on the amount of Ca taken up by the stores. Filled circles show the results when caffeine+IP_3 was used as the assay solution, while open circles were obtained with caffeine assay. The arbitrary unit of the ordinate corresponded to 125 μmoles of Ca /liter cell water (see the legend). The maximum capacity was about 170 μmoles/liter cell water and was obtained at near pCa 6.

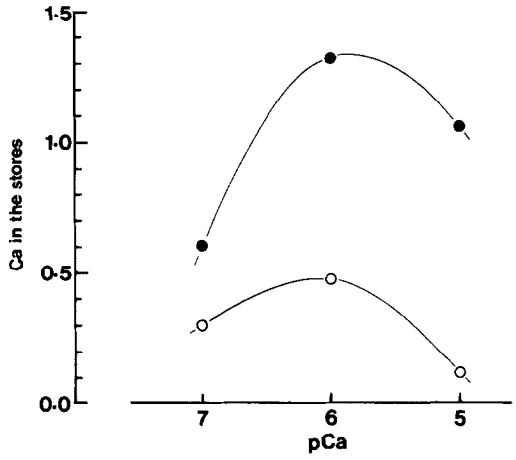

Fig. 2. pCa dependence of the Ca loading. A fibre bundle was loaded with Ca (buffered with 10 mM EGTA) at the pCa indicated for 360 s and the assay was carried out either by caffeine (open circles) or by caffeine+IP_3 (filled circles). 1.0 in the ordinate corresponds to the Ca response for caffeine +IP_3 assay after Ca loading at pCa 6 for 120 s, and can be roughly converted into an absolute value taking the following into consideration: calibration of fura-2, the ratio of the cross-sectional area of the preparation to that of the capillary minus the metal wire, the volume ratio of cell water to the whole preparation (taken as 0.5) and the leakage during the period between the end of Ca loading and the assay (33 %, cf. legend of Fig. 3).

Caffeine releasable Ca was always less than a half of the total Ca in the stores, and declined at the higher Ca^{2+} concentration with a peak round pCa 6. As suggested by the biphasic dependence, the caffeine releasable store has a CICR mechanism similar to that found in skeletal muscle (9), and the mechanism is activated at pCa below 6 and is enhanced by caffeine and adenine nucleotides (in preparation). Thus, caffeine seems to release Ca from the store through an enhancement of the CICR mechanism as in skeletal muscle (9). The difference between the two curves in Fig. 2, i.e. the amount of Ca released by caffeine+IP_3 but not by caffeine alone, monotonically increased with the Ca^{2+} concentration. Above results imply that there are two classes of stores; one half of them (caffeine releasable store) has both Ca- and IP_3-induced Ca release mechanisms and the rest only the latter.

To further investigate the IP_3-induced Ca release, experiments shown in Fig. 3A were designed. Between the Ca loading and the assay, a test procedure was inserted, i.e. fibre bundles were treated with 10 μM IP_3 at two Ca concentrations in the absence of Mg and ATP for various lengths of time. The decrease in the Ca response at the assay indicated the amount of Ca

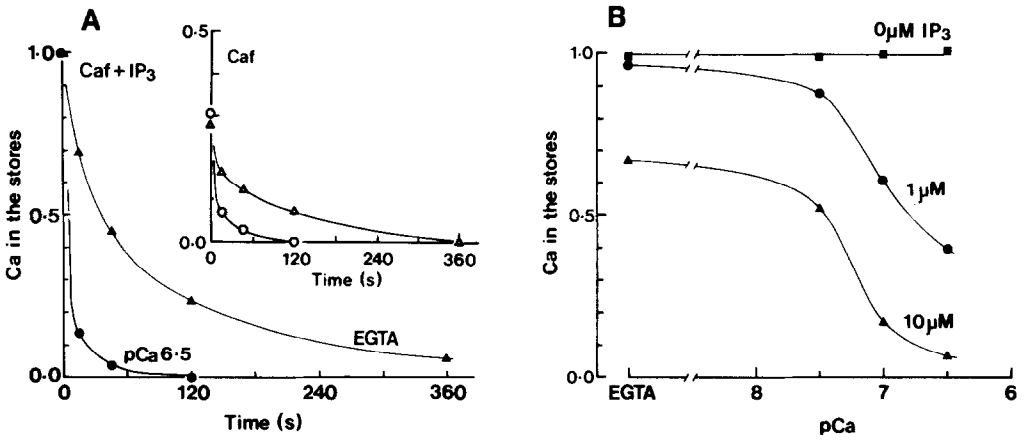

Fig. 3. A, Time course of the Ca release induced by 10 μ IP_3 at two Ca^{2+} concentrations in the absence of Mg and ATP. Triangles 10 mM EGTA and no Ca, circles pCa 6.5. Filled symbols obtained by caffeine+IP_3 assay while open symbols in the inset by caffeine assay. Even without IP_3 there was a slow decline of Ca (leakage) with a rate constant of about 0.1 min^{-1}. B, pCa dependence of the Ca release induced by 15 s application of 0 (squares), 1 (circles), 10 (triangles) μM IP_3. Caffeine+IP_3 assay. 1.0 in the ordinate corresponds to no test treatment.

released during the test procedure. Since ATP was absent during the test to stop Ca uptake, Ca release process could be singled out by this protocol. At pCa 6.5, IP_3 induced rapid and complete Ca release from the caffeine-releasable store as well as from the whole stores. In the virtual absence of Ca^{2+}, the rate of Ca release induced by IP_3 was much slower than that at pCa 6.5. In Fig. 3B the amount of Ca remaining in the stores after 15 s treatment with 0, 1 or 10 μM IP_3 is plotted against the Ca^{2+} concentration during the IP_3 application. The initial rate of IP_3-induced Ca release is markedly dependent on pCa round 7 where the contractile system is relaxed (7), but Ca^{2+} itself had no effect in this range.

Ca^{2+} dependent metabolism of IP_3 (10,11) dose not affect the results. For IP_3 was applied in the absence of Mg and ATP, neither IP_3 phosphatase which requires Mg to be active (12) nor IP_3 kinase could work under the experimental condition. Ca^{2+} dependence of the *amount* of IP_3-induced Ca release has been reported in smooth muscle cells, and Ca^{2+} had little effect below 1 μM (2,3) but the *rate* of release has not been mentioned.

The Ca^{2+}-dependence of the rate of IP_3-induced Ca release may constitute an important factor, if IP_3 is indeed a physiological signal for the Ca

release from the intracellular stores. For example, the rate of Ca release might be modulated by the resting Ca^{2+} concentration or by the influx of Ca^{2+} from outside. Heterogeneity of Ca stores in smooth muscle has been suggested in cultured cells but in a somewhat different way (3). Further studies are needed to clarify if the functional heterogeneity corresponds to the difference in the structure and if the different stores are utilized in response to different stimuli.

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