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USE OF RYANODINE FOR FUNCTIONAL REMOVAL OF THE CALCIUM STORE IN SMOOTH MUSCLE CELLS OF THE GUINEA-PIG

M. Iino, T. Kobayashi and M. Endo

Department of Pharmacology, Faculty of Medicine,
University of Tokyo, Tokyo 113, Japan

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Summary Calcium store of the skinned fibers of the guinea-pig portal vein, pulmonary artery and taenia caeci consisted of two classes: one with both Ca-induced Ca release (CICR) and inositol 1,4,5-trisphosphate (IP_3)-induced Ca release (IICR) mechanisms ($S\alpha$) and the other only with IICR mechanism ($S\beta$). Ryanodine, applied during the CICR was activated, locked the CICR channels open, but the drug had practically no effect on the IICR mechanism. Thus, after the ryanodine treatment the Ca store with the CICR ($S\alpha$) lost its capacity to hold Ca. Changes in the agonist-evoked contraction of intact muscle due to the ryanodine treatment suggest that agonists release Ca from $S\alpha$ which produces the initial phase of contractures.

Ryanodine, a plant alkaloid, has been shown to act on the Ca release channels of the fragmented sarcoplasmic reticulums of skeletal muscle and to lock the channels in an open state (1,2). Thus after ryanodine treatment Ca is kept released from the sarcoplasmic reticulum, and the resulting increase in the intracellular Ca concentration explains contracture of the muscle, a well-known effect of the drug on skeletal muscle. However, the mechanism of the effect of the drug on smooth muscle has not been well elucidated. In the present study, the effect of ryanodine on the smooth muscle Ca store was examined using skinned fibers of guinea-pig taenia, portal vein and pulmonary artery. The results showed that the drug locked the CICR channels open in accordance with its effect on the skeletal muscle counterpart, but it had no effect on the IICR mechanism. The discriminating effect of the drug was used in the intact smooth muscle experiments to determine the physiological significance of the ryanodine-sensitive Ca store in agonist-induced contractures.

ABBREVIATIONS. CICR, Ca-induced Ca release; IP_3, inositol 1,4,5-trisphosphate; IICR, IP_3-induced Ca release; EGTA, ethyleneglycol-bis(β-aminoethyl ether) N,N,N',N'-tetraacetic acid.

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METHODS

Thin fibre bundles, widths 150-250μm for taenia caeci or thickness ~70μm and width ~300μm for vascular smooth muscles, were carefully obtained from the respective tissues of guinea-pig. Skinned fibre experiments. Methods have been described elsewhere (3). Briefly, fibre bundles were chemically skinned by saponin (50μg/ml, 30 min) in a relaxing solution and were placed in a capillary cuvette. Ca released from the fibre upon application of caffeine and/or IP_3 was measured using microfluorometry of fura-2. Tension measurement. Intact fibre bundles were held horizontally, one end being connected to a straingauge transducer (AE801, Akers). Solution change was accomplished by transferring the preparation from a well to another each containing an appropriate solution. Experiments were carried out at 20-22℃.

RESULTS AND DISCUSSION

Figure 1A-C compares the Ca release from the store in a skinned fiber of taenia upon application of either 50 mM caffeine (upper traces) or 10μM IP_3 (lower traces) before and after ryanodine treatment. Before each trace, the store had been loaded with Ca, at pCa 6 for 180 s in the presence of 4 mM MgATP. ATP then was washed to avoid reuptake of Ca during subsequent Ca release, so that the amount of Ca released indicated the maximum amount of Ca releasable under each condition. It has been shown (3) that the Ca store of

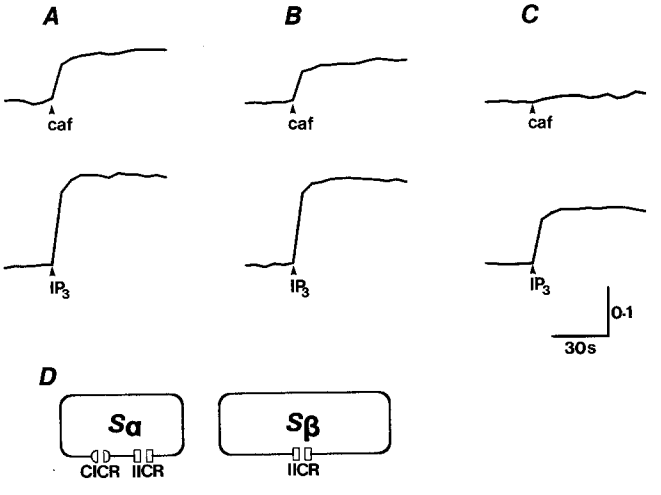

Fig. 1 A-C, Ca dependent fluorescence intensity change of fura-2 (30.5μM) immersing a skinned fiber of taenia. Ca was released from the store by the application at the arrow head of either 50 mM caffeine (upper traces) or 10μM IP_3 (lower traces). A, Control. B, After treatment of 30μM ryanodine with 10μM IP_3 at pCa 7 for 120 s. C, After additional treatment of ryanodine with 50 mM-caffeine at pCa 5.7 for 120 s. Vertical bar indicates 0.1 of the maximum fluorescence change. D, Scheme of compartments of the Ca store in smooth muscle cells.

taenia consists of two classes as schematically shown in Fig. 1D. A part of the store ($S\alpha$) responds to both caffeine and IP_3. Since caffeine releases Ca by enhancing the CICR, $S\alpha$ has both CICR and IICR mechanisms. The rest of the store ($S\beta$) responds only to IP_3, and it lacks the CICR mechanism. In accordance with this notion, the amount of Ca released by the application of IP_3 (or IP_3+caffeine) was about twice as large as that by caffeine alone before the ryanodine treatment (Fig. 1A). Qualitatively similar heterogeneity was found in other smooth muscles, although the fraction of $S\alpha$ was different: about 40%, 5% and 60% in taenia caeci, portal vein and pulmonary artery, respectively.

When ryanodine was applied for 120 s, between A and B, in the presence of 30μM IP_3 and 100 nM Ca^{2+}, a condition that would strongly activate the IICR mechanism (3), it failed to show any appreciable after-effect (Fig. 1 A vs. B). Neither did treatment with ryanodine in the absence of both Ca and IP_3 show any effect (not shown). However, if 30μM ryanodine was applied again, between B and C, for 120 s in the presence of Ca (pCa 5.7) and 50 mM caffeine to activate the CICR, the caffeine-releasable fraction decreased to almost null and the amount of Ca released by IP_3 decreased by some 40% (A vs. C). The same results have been obtained with 1μM ryanodine and 10 mM caffeine at pCa 5.7. These results suggest that the CICR channels in smooth muscle were locked open by ryanodine as in skeletal muscle, and the Ca store with this Ca release mechanism ($S\alpha$) lost its capacity to hold Ca inside. The results also show that ryanodine acts on the CICR channels only when they are open, and that it does not have any appreciable effect on the IICR channels even if the drug is applied when these channels are open. Similar results have been obtained in skinned fibre bundles of the vascular smooth muscles. We also found that the effect of ryanodine was practically irreversible at 20°C and the traces shown in Fig. 1 B and C were in fact obtained after ryanodine had been washed. However, if the fiber was washed at 37°C, the effect of ryanodine could be mostly removed in half an hour or so.

Then we studied effects of ryanodine on contractions of intact muscle preparations, because the discriminating action of ryanodine may be useful as a tool to remove the function of a certain part of the intracellular Ca store (Sα) in intact cells. We could observe contractions repeatedly evoked either by a high-K solution or by an agonist (carbachol or noradrenaline) in the continued presence of 30μM ryanodine. The first caffeine (25 mM) contracture in the presence of 30μM ryanodine was exactly the same as that in the absence of the drug. However, the following applications of caffeine almost failed to elicit contraction even after the withdrawal of ryanodine, albeit the muscle can still respond with a contraction to a high-K solution. These results suggest that ryanodine acts on the CICR channels only when they are open (as the result of the caffeine application) and lock them in an open state as in skinned fibers. Inability of K- and agonist-contractures to make ryanodine effective suggests that the CICR is only poorly activated in these contractures as compared with caffeine contractures, although the magnitude of the contractions was similar or even greater than that with caffeine. Unlike in skeletal muscle, smooth muscles did not show irreversible contracture after the ryanodine treatment. This is probably because released Ca was extruded from the cell as suggested by the ^{45}Ca efflux experiment (4) or sequestered by a ryanodine-insensitive Ca pool. The effect of ryanodine stayed at least a few hours after the withdrawal of the drug at 20℃, but was mostly removed after an incubation at 37℃ for 40 min or so.

Figure 2 shows the tension records of intact fiber bundles. The left hand side and the middle traces were obtained before and after ryanodine treatment, respectively. The ryanodine treatment was carried out with 30μM of the drug in the following sequence: 4 min pretreatment, 2 min treatment in the presence of 25 mM caffeine and removal of both agents. The right hand side traces show the difference between the tension traces before and after the ryanodine treatment (left - middle). Caffeine contractures in taenia are shown in A and was greatly diminished after the ryanodine treatment as already described. Similar results were obtained in both portal vein and pulmonary

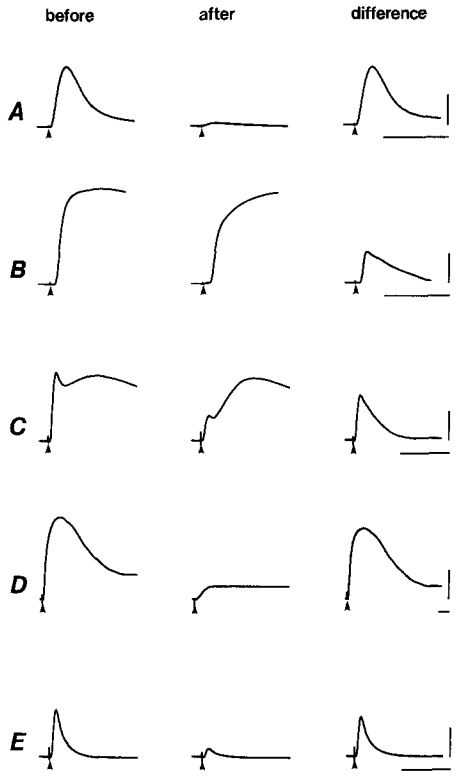

Fig. 2 Tension traces of intact smooth muscle bundles before (left) and after (middle) the treatment of 30μM ryanodine with 25 mM caffeine. Traces on the right represent the differences of tension traces (left - middle). Contractions were evoked by 25 mM caffeine (A, taenia), 10μM carbachol (B, taenia), or 10μM noradrenaline (C and E, portal vein; D, pulmonary artery). A-D, 2 mM extracellular Ca. In E Ca was omitted from the bathing solutions and 5 mM EGTA was added. Caffeine and agonists were applied at the arrow heads. Horizontal calibrations, 60 s. Vertical bars indicate 1mN (A, B, C, E) or 0.1 mN (D).

artery. 10μM carbachol-evoked contractions of taenia are shown in B. The lag time between the agonist application and the rise of tension was increased, the rate of tension rise was slower but the peak tension was almost the same. These changes are reflected in the difference trace which has a sharp rise and a slow return to near zero. 10μM noradrenaline-induced contraction in portal vein (C) had an initial transient peak followed by a sustained plateau of a similar magnitude. The ryanodine treatment seemed to affect mainly the initial component. Noradrenaline (10μM) evoked a phasic contraction in pulmonary artery strip (D). It was accompanied by a tonic component of variable size, but usually the tonic component had a much smaller magnitude than the phasic one. The ryanodine treatment seemed to suppress mainly the phasic component. It should be kept in mind that the difference in

the tension traces would not be linearly related to the amount of Ca mobilized. Even so, it seems reasonable to conclude from the above experiments that Ca release from the ryanodine sensitive store ($S\alpha$) participates at least in the initial phase of the agonist-evoked contractions and this is quite important in pulmonary artery although its contribution is less in taenia and in portal vein.

It is well known that agonists evoke transient contractions of smooth muscle in the absence of extracellular Ca (5) as reproduced here for the case of portal vein (Fig. 2E left). This seems mainly due to Ca release from the ryanodine sensitive component ($S\alpha$), because after the ryanodine treatment the agonist-induced contraction in portal vein was greatly diminished (Fig. 2E middle) even though $S\alpha$ occupied only 5% of the total store in this muscle. The suppression of the contraction was almost complete in taenia and pulmonary artery (not shown). Results obtained in skinned fibers suggest that ryanodine would have depleted only a part of the Ca store ($S\alpha$) and there was a considerable amount of store ($S\beta$) left which had the IICR mechanism. If the second messenger for the agonist is indeed IP_3 as has been suggested in many other cells (6), then why agonists did not release Ca from $S\beta$ in the absence of extracellular Ca? One of the possibilities is that either IP_3 does not reach $S\beta$ in agonist-induced contractions or IP_3 is not used as the second messenger. Another possibility is that the IICR in $S\beta$ requires extracellular Ca. Since the IICR has been shown to be dependent on Ca concentration near pCa 7 (3), it is possible that IP_3 releases Ca from $S\beta$ only when there is sufficient Ca influx to keep the Ca concentration in the vicinity of the store higher than a certain critical level. There could be certainly other possibilities and this important problem awaits further clarification.

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