100. Calcium-Induced Calcium Release and "Depolarization"-Induced Calcium Release: their Physiological Significance

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How depolarization of the T-system leads to the release of calcium from the sarcoplasmic reticulum (SR) is the least understood process in excitation contraction coupling in skeletal muscle. Experiments on the SR in skinned muscle fibres1 have suggested at least two possible mechanisms for the process. For example, since calcium ion itself was shown to induce a release of calcium from the SR,2-5 it is conceivable that depolarization of the T-system might cause calcium to enter from outside or calcium bound to the membrane of the T-system to be released, the amount of which may be too small to activate the contractile system by itself but still it acts on the SR to induce the release of more calcium from the latter structure. Alternatively, since "depolarization" of the SR membrane was also shown to produce a release of calcium,6 depolarization of the T-system might somehow cause a "depolarization" of the SR, which in turn acts as the final trigger for release of calcium. It has not yet been clear, however, whether these two kinds of stimuli for the SR are independent, or they are mutually interrelated. For example, calcium ion may induce a release of calcium by "depolarizing" the SR membrane, or conversely the main fraction of calcium released by "depolarization" may be derived from the secondary release process evoked by a small amount of calcium that is directly released by "depolarization". It is, therefore, important to examine whether these two stimuli are independent or not, and if they are, which is more important physiologically. We have found7,8 that two stimuli are practically independent, and that the hypothesis that calcium ion is the primary mediator of transmitting information from the T-system to the SR to induce the release of calcium is rather unlikely.

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Methods. Experiments were done essentially in the same way as previously described.4,6,9

Results. The effect of calcium ion and that of "depolarization" to produce release of calcium showed several different characteristics from each other. Firstly, whereas calcium-induced calcium release is strongly affected by free magnesium ion concentration,9 "depolarization"-induced release of calcium is not influenced by a change in the level of free magnesium. Figure 1 shows the amount of remaining calcium after "depolarizing" the SR, which had been preloaded to a fixed level, by replacing various fractions of methanesulfonate with chloride under two different concentrations of free magnesium. It is clearly seen that with the increase in the fraction of chloride replacement, the amount of remaining calcium decreased, which indicated that the amount of calcium released during the ionic replacement increased. It is also seen that no appreciable difference in the response to chloride was observed when free magnesium was reduced from 0.9 mM to 0.05 mM, in sharp contrast to a strong enhancement of calcium-induced calcium release by the same reduction of free magnesium.9 In these experiments, replacement with chloride was made in the presence of 10 mM EGTA, which prevented a rise in free calcium ion concentration while calcium was being released by "depolarization". Otherwise, a rise in free calcium ion concentra-
Ca, "Depolarization" and Ca Release

The release by "depolarization" might have caused a further release of calcium, and since the secondary release process is subject to the influence of the level of free magnesium, the overall results might have been affected by free magnesium.

Secondly, in the case of calcium-induced calcium release, a certain level of preloading of the SR is required, and above this level, the more the SR was loaded, the greater fraction of calcium was released by a fixed concentration of stimulating free calcium. However, as shown in Fig. 2, there seems to be no such loading requirement necessary for "depolarization"-induced release of calcium, and the amount of calcium released by "depolarization" is more or less a constant fraction of the amount existing immediately before the "depolarization".

Thirdly, different substances separately inhibit either calcium-induced release or "depolarization"-induced release of calcium, without appreciably affecting the other process. Ford and Podolsky found that procaine effectively inhibited the calcium-induced calcium release mechanism. It was confirmed by experiments as illustrated in Fig. 3A. The experiments were very similar to those of Fig. 2 in the accompanying paper. In these experiments, the level of free magnesium used was 0.05 mM, and hence, $10^{-5}$ M of free calcium.
This releasing effect of calcium was completely inhibited by 10 mM procaine, as shown in the figure. In contrast to this, procaine showed no inhibition at all for the "depolarization"-induced release of calcium as shown in Fig. 3B, or even it potentiated slightly as indicated by the slightly smaller amount of remaining calcium in the figure. On the other hand, 40 mM or higher concentrations of sucrose, or many other sugars like glucose, fructose or xylose and so on, completely inhibited "depolarization"-induced release of calcium, whereas the same concentration of sugars showed little effect on calcium-induced release of calcium, although a slight inhibition was observed under a certain condition.

Discussion. The differences in characteristics of calcium-induced release and "depolarization"-induced release of calcium de-
The effect of procaine on caffeine contracture (A) and on potassium contracture (B) of an intact single fibre. Fibre 40719.

A. Maximum tension developed by the fibre during 1 min period after each application of caffeine was plotted against concentration of the drug. Caffeine sensitivity of this fibre in the absence of procaine was higher than usual.

B. Maximum tension evoked by each concentration of potassium was plotted. Potassium-chloride product was kept constant, Tris and methanesulfonate being used as substituting ions. Maximum tension evoked by 120 mM K after caffeine contracture series was exactly the same as before the series.

scribed above suggest that these two stimuli are independent from each other.

To assess the physiological significance of the calcium-induced calcium release mechanism, procaine can be used as a very convenient tool. Since the drug inhibits calcium-induced release of calcium, if physiological activation mechanism is primarily mediated by calcium ion, the drug should also inhibit the physiological activation. However, Heistracher and Hunt's experiments showed that procaine does not seem to inhibit the physiological activation mechanism of intact fibres. On the other hand, it has long been known that caffeine contracture of intact muscle fibres is blocked by procaine. This is in agreement with the mechanism of action of caffeine as the acceleration of calcium-induced calcium release. These effects of procaine on intact fibres were confirmed by the experiments as illustrated in Figs. 4A and 4B, done on one and the same single fibre. While caffeine contracture was strongly inhibited by 10 mM procaine (Fig. 4A), no inhibition, or even slight potentiation, was observed on potassium contracture (Fig. 4B). Since procaine was certainly exerting its effect on this fibre as evidenced by the inhibition of the
caffeine contracture, the fact that potassium contracture was not inhibited by procaine indicates that calcium ion is not the primary mediator of the information from the T-system to the SR. This conclusion is in agreement with that from an independent assessment.13)

In contrast to the calcium-induced release of calcium, results so far obtained by skinned fibre experiments (ref. 6 and this paper) are consistent with the hypothesis that “depolarization” of the SR is the direct cause of physiological release of calcium, although they do not prove it.

It should be added that in spite of the above conclusion, calcium-induced calcium release mechanism may help to release more amount of calcium from the SR, under certain circumstances; for example, under the influence of a low concentration of caffeine.13) This may well be the explanation of twitch potentiating effect of a low concentration of caffeine. The situation in cardiac or smooth muscle may also be different. In these muscles, the SR may well be more sensitive to the effect of calcium to release calcium. Therefore, the physiological role of the calcium-induced calcium release mechanism should be examined properly in each preparation, before any conclusion is drawn about the activation mechanism of these kinds of muscle.

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