The Effects of Ryanodine on Calcium-Overloaded Sheep Cardiac Purkinje Fibers

M. Valdeolmillos and D. A. Eisner
From the Department of Physiology, University College London, Gower Street, London, United Kingdom

SUMMARY. Prolonged exposure to high concentrations of strophanthinidin produces an initial increase followed by a subsequent decrease of twitch tension. The slow decrease is termed calcium overload. The aim of the present work was to investigate the effects of ryanodine (an inhibitor of calcium release from the sarcoplasmic reticulum) on calcium-overloaded sheep cardiac Purkinje fibers. The fibers were voltage-clamped, and tension was measured while monitoring the intracellular calcium concentration with the photoprotein aequorin. When strophanthinidin (10 μM) was applied to produce calcium overload, a depolarizing pulse produced twitch, and tonic components of tension and repolarization produced an aftercontraction. These components of tension were accompanied by corresponding increases of aequorin light. Ryanodine (1 μM) gave a transient increase of twitch tension. The twitch then decreased to very low levels. The aftercontraction and its corresponding aequorin light signal decreased monotonically on application of ryanodine. It has been suggested that the fall of force in calcium overload may be due to random diastolic release of calcium from the sarcoplasmic reticulum interfering with subsequent systolic calcium release. We suggest that the positive inotropic effect of ryanodine can be explained if ryanodine decreases the diastolic release of calcium. The transient positive inotropic effect of ryanodine reported here is therefore consistent with the hypothesis that the fall of force in calcium overload is due to diastolic calcium oscillations. (Circ Res 56: 452-456, 1985)
potential (mV)

-20

-70

tension

10 uN

5 min

FIGURE 1. The effects of strophanthidin and ryanodine on the contraction of a voltage-clamped cardiac Purkinje fiber. Traces show: top, membrane potential; bottom, tension (0.1–10 Hz). Strophanthidin (10 μM) and ryanodine (1 μM) were applied for the periods shown. Throughout the experiment, the membrane potential was held at −68 mV, and a 1-second duration pulse to −20 mV was applied at 0.33 Hz.

This paper shows that ryanodine produces a transient increase of twitch tension. When changing solutions with a conventional valve, it is often difficult to avoid artefacts due to transient changes of solution level, temperature, etc. Ryanodine was therefore added as a concentrated solution directly to the bottle containing the superfusing solution. This produced a delay due to the length of the tubing which has been allowed for.

In the present experiments, [Ca^{2+}] was measured with the photoprotein aequorin. This was microinjected into 20–30 cells (see Wier and Isenberg, 1982; Blinks et al., 1982; Allen and Orchard, 1983, for further details). The light emitted from aequorin was collected by a photomultiplier tube mounted below the bath and connected to the base of the bath (made from a coverslip) by a Lucite light guide. A shutter could be closed in front of the photomultiplier to correct for background counts (Cannell and Allen, 1983). To minimize the dark counts from the photomultiplier tube, the output from the tube was connected to a discriminator amplifier (Princeton Applied Research 1121A). The records shown were obtained from a rate meter and were averaged from several voltage clamp pulses, using a microprocessor.

The experimental solutions contained (in mM): NaCl, 145; CaCl₂, 5; MgCl₂, 2; KCl, 5; glucose, 10. It was buffered with 5 mM Tris-HEPES (pH 7.3). All experiments were performed at 35 ± 1°C. Strophanthidin (Sigma) was kept as a stock solution (10⁻² M in ethanol) and was added as required. Ryanodine was a generous gift of Dr. J. Kenyon (University of Texas at Dallas). It was kept frozen in a solution of 10 mM in H₂O.

Results

Figure 1 shows a record from an experiment in which a voltage-clamped sheep cardiac Purkinje fiber was exposed to strophanthidin for a prolonged period. This produced an increase of force which developed over a period of about 5 minutes, and then subsequently declined. This secondary fall of force has been observed previously and has been termed Ca^{2+} overload (Vassalle and Lin, 1979). At this stage, ryanodine (1 μM) was added. This produced an initial rise of tension which lasted for 2 minutes before tension eventually fell to a low level. Control experiments showed that, as expected, if ryanodine was added in the absence of strophanthidin, there was no initial increase of tension and the twitch declined monotonically. Furthermore, if ryanodine was added in the presence of strophanthidin before the twitch had begun to decline, there was no increase of twitch. The effects of ryanodine were examined in seven fibers which were Ca^{2+} overloaded, as judged by the fact that twitch tension had begun to decline. In five of these, there was a transient increase of tension which had a mean value of 42 ± 6% (SEM). In the remaining two experiments, there was no significant increase of twitch tension and the twitch declined slowly.

The time courses of onset and decay of the positive inotropic effect of ryanodine are shown in more detail in Figure 2, which is taken from a similar experiment. The preparation had been exposed to strophanthidin for 45 minutes at the start of the record shown. When ryanodine was subsequently added, the twitch first increased, before decreasing to a very low value. In this experiment, the photoprotein aequorin had been previously injected into the fiber in order to measure [Ca^{2+}]. Specimen aequorin and tension records from this experiment are shown in Figure 3. Figure 3A shows a control record obtained before the exposure to strophanthidin shown in Figure 2. Depolarization produces a small increase of [Ca^{2+}] and tension. The other records were obtained at the times indicated on Figure 2. The addition of strophanthidin (panel B) increased the twitch tension and produced a component of tonic tension which developed during the depolarizing pulse. Repolarization produced oscillatory aftercontractions. The various components of tension are accompanied by corresponding changes of [Ca^{2+}] as shown by the aequorin light. In particular, it should be noted that the diastolic level of
Ryanodine

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**Figure 2.** Time course of onset of the effects of ryanodine on a Ca**++**-overloaded cardiac Purkinje fiber. Traces show: top, membrane potential; middle, tension (0.1-10 Hz); bottom, aftercontraction magnitude as measured from an unfiltered tension record. The preparation had been exposed to strophanthidin (10 μm) for 45 minutes before the beginning of the record shown. Ryanodine (1 μm) was applied for the period indicated. The membrane potential was held at -80 mV and a 500 msec duration pulse to -20 mV was applied at 0.33 Hz.

Aequorin light emission is increased, and that, following the transient increase, light falls to below the diastolic level before increasing during the pulse. This undershoot of aequorin light in Ca**++** overload has been reported previously (Orchard et al., 1983; Wier and Hess, 1984) and appears to result from a temporary decrease of spontaneous calcium oscillations following stimulation (Stern et al., 1983). Figure 3C was obtained during the period of increased twitch tension shortly after ryanodine was added. It is clear that, although the twitch is increased, the aftercontraction is smaller than that shown in Figure 3B. Furthermore, the second aftercontraction which was present in panel B is now abolished. There is a corresponding decrease in the oscillations of [Ca**++**]. It should also be noted that the increase of twitch tension is accompanied by a decrease of the peak aequorin light. Figure 3D shows the effects of prolonged exposure to ryanodine. The twitch and aftercontraction have been completely abolished, as have the corresponding changes of [Ca**++**]. Depolarization now produces only a tonic increase of tension. The changes of aftercontraction magnitude are plotted below the tension record of Figure 2. It is evident that the aftercontraction begins to decline at almost exactly the same time as the twitch increases.

**Discussion**

The present work shows that, in Ca**++**-overloaded Purkinje fibers, ryanodine produces a transient increase of twitch tension. This transient increase of twitch tension occurs at the same time as the aftercontraction starts to decrease. The transient positive inotropic effect of ryanodine is seen only in preparations that have been exposed to strophanthidin long enough for the twitch to decline (i.e., Ca**++** overloaded). As previously reported (Sutko and Kenyon, 1983), when ryanodine is applied before Ca**++** overload occurs, only a fall of twitch tension is seen. Although the negative ionotropic effect of Ca**++** overload produced by prolonged exposure to strophanthidin is well known, there is no agreed explanation for its cause. Recent work has shown that the decrease of force is not accompanied by a

**Figure 3.** Specimen records of the effects of strophanthidin and ryanodine on aequorin light and tension. Data from the experiment of Figure 2. In each panel, traces show: top, membrane potential; middle, aequorin light; bottom, tension. All records are the average of 16 pulses. Panels show: A, control, obtained before the application of strophanthidin shown in Figure 2; B, after 48 minutes of exposure to strophanthidin; C, 3 minutes after adding ryanodine (1 μm); D, 7 minutes after adding ryanodine (1 μm). The points at which records B-D were obtained are shown in Figure 2.
decrease of systolic [Ca++] as measured by aequorin (Wier and Hess, 1984; Allen et al., 1984). The present work shows that the transient increase of force produced by ryanodine is not accompanied by a rise of [Ca++]. It is therefore likely that the positive inotropic effect of ryanodine is related to the mechanism of the negative inotropic effect of Ca++ overload, since in neither case does force change in the same direction as peak systolic aequorin light.

Two explanations have been suggested for the fall of force in Ca++ overload. (1) The sensitivity of the contractile apparatus to [Ca++] may be decreased during the period of Ca++ overload. For example, it is known that exposure to strophanthidin decreases intracellular pH (Deitmer and Ellis, 1978; Vaughan-Jones et al., 1983) and this acidification will decrease force (Fabio and Fabio, 1978; Allen and Orchard, 1983). (2) The decrease of systolic force may be due to the spontaneous diastolic Ca++ oscillations which are present in strophanthidin intoxicated preparations. Kort and Lakatta (1984) have suggested that these oscillations will deplete the sarcoplasmic reticulum of Ca++ and thereby interfere with systolic Ca++ release. Since the oscillations can be out of phase in different cells, the systolic Ca++ release will vary from cell to cell. The less activated cells will add an extra compliance which will allow the more activated ones to shorten, thus decreasing the force produced by the preparation (Eisner et al., 1984a). On the other hand, if the mean systolic [Ca++] remains constant, the increased variance of systolic [Ca++] will increase the peak systolic aequorin light, since the light emission is approximately proportional to [Ca++]^2. Furthermore, even if the mean systolic [Ca++] decreases, if its variance increases sufficiently, the mean aequorin signal will increase. Therefore, diastolic Ca++ oscillations may be able to decrease total systolic force even though the mean Ca++ signal as detected by aequorin increases (Eisner et al., 1984a). A similar discrepancy between changes of light and tension has been discussed previously (Orchard et al., 1983; Wier et al., 1983). It is worth reemphasizing that the above considerations mean that the aequorin light records of Figure 3 cannot be interpreted simply as being related to the mean [Ca++]

If the fall of force in Ca++ overload is due to Ca++ oscillations, it is easy to see how ryanodine can increase the twitch tension. Ryanodine will decrease SR Ca++ release, and will therefore decrease the Ca++ oscillations. This reduction of oscillations also leads to the reduction in the aftercontraction magnitude. The effects of ryanodine on the twitch will be the combination of two factors. First, ryanodine will decrease the diastolic Ca++ oscillations and thereby increase the systolic release of Ca++ and, hence, the twitch. Second, ryanodine will directly decrease the Ca++ release that produces the twitch. The balance of these two factors could produce the phasic increase of twitch tension seen in the present work. The transient positive inotropic effect of ryanodine is therefore consistent with the hypothesis that the fall of force in Ca++ overload is due to diastolic Ca++ oscillations. Furthermore, if in some cases the two factors cancel out, one can explain the lack of an initial positive inotropic effect in two of the overloaded fibers studied.

It is also necessary to consider whether the present results can be accounted for by other explanations. For example, the acidification produced by Ca++ overload has been shown to be due to an increase of [Ca++] (Vaughan-Jones et al., 1983). Therefore, the removal of these Ca++ oscillations by ryanodine may well produce an intracellular alkalinization and thence increase force. One problem with this hypothesis, however, is that an intracellular alkalinization will increase not only the twitch, but also the tonic tension (Eisner et al., 1982). Therefore, this hypothesis cannot explain the result of Figure 3, B and C, where ryanodine increases twitch tension with no increase of tonic tension. This lack of an increase of tonic tension also excludes other explanations in which the transient increase in twitch tension produced by ryanodine is ascribed to an increase of Ca++ sensitivity of the contractile apparatus. It should, however, be noted that these results do not exclude the possibility that a part of the fall in force of Ca++ overload is due to an intracellular acidification.

In conclusion, we suggest that the positive inotropic effect of ryanodine seen in Ca++-overloaded preparations involves a removal of the Ca++ oscillations which may produce the fall of tension in Ca++ overload.

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Address for reprints: D. A. Eisner, University College London, Gower Street, London WC1E 6BT, United Kingdom.

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References


Deitmer JW, Ellis D (1980) Interactions between the regulation of the intracellular pH and sodium activity of sheep cardiac Pur


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