Br. J. Pharmac. (1984) **81**, 013–015

Ryanodine prolongs Ca-currents while suppressing contraction in rat ventricular muscle cells

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Ryanodine (1 μM) suppressed or abolished contraction in response to step depolarizations in voltage-clamped cells isolated from adult rat ventricular myocardium. The step depolarizations evoked the second inward current, which is carried largely by Ca ions under these conditions, and there was little or no change in the amplitude of this current when contraction was reduced or abolished by ryanodine. The effects of ryanodine on contraction were, however, accompanied by a prolongation of the second inward current resulting from a slowing of the apparent inactivation of this current. It is suggested that ryanodine affects steps in excitation-contraction coupling subsequent to the second inward current, perhaps Ca-release from intracellular stores, and that this slows a Ca-dependent inactivation of second inward current.

Introduction Ryanodine is a neutral alkaloid which exerts a negative inotropic effect on mammalian cardiac muscle (Jenden & Fairhurst, 1969). It has been suggested that the mechanism of action involves a reduction in release of Ca from internal stores (Sutko, Willerson, Templeton, Jones & Besch, 1979; Sutko & Willerson, 1980; see Penefsky, 1974). We have investigated the action of ryanodine on the second inward current carried by Ca (Powell, Terrar & Twist, 1981; Mitchell, Powell, Terrar & Twist, 1983) and on contraction in single cells isolated from rat ventricular muscle.

Methods Cells were isolated from ventricular muscle of adult rats by a procedure using collagenase (Powell, Terrar & Twist, 1980). A single electrode voltage-clamp (Dagan 8100) was used to record the second inward current, and the accompanying contraction was monitored with a photodiode mounted in the microscope eyepiece to collect light from the cell under study (Powell *et al.*, 1981; Mitchell *et al.*, 1983; see Kass, 1981). The temperature of the solution bathing the cells was controlled at 36°C. The solution contained (mM): NaCl 118.5, $NaHCO_3$ 14.5, $CaCl_2$ 2.5, KCl 2.6, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 11.1, and 5 mg ml^{-1} bovine serum albumin (Pentex Fraction V. Miles Laboratories). Ryanodine was a gift from Merck Sharp and Dohme.

Results Figure 1a shows the second inward current and the accompanying contraction during a step depolarization from −40 mV to 0 mV. Under these conditions the second inward current (I_{si}) is carried largely by Ca (Mitchell *et al.*, 1983). When ryanodine (1 μM) was applied in the external solution, there was a gradual reduction of the amplitude of contraction during successive step depolarizations (0.3 to 0.5 Hz), until after approximately 2 min the contraction was greatly suppressed or abolished (Figure 1a).

When the contraction was suppressed by ryanodine, there was little or no change in the amplitude of I_{si}, but the time course of I_{si} was prolonged (Figure 1a). This prolongation is shown more clearly in Figure 1b which shows the amplitude of I_{si} plotted semilogarithmically against time after peak inward current. It can be seen that before ryanodine the decline, or apparent inactivation, of I_{si} could be described as the sum of two exponentials (see also Mitchell *et al.*, 1983). After prolongation of I_{si} by ryanodine, the decline could be fitted by the same two exponentials, but with the relative contributions of the two components changed in favour of the slow exponential. It should be mentioned that under these conditions, there is no significant contribution to the recorded I_{si} by K currents which are sensitive to 4-aminopyridine or Cs, and that the apparent inactivation cannot result from these (Mitchell *et al.*, 1983). Thus, the reduction in transient outward current by ryanodine found for Purkinje fibres by Kenyon & Sutko (1983) appears to be an additional effect of ryanodine.

If inactivation of I_{si} has indeed been modified by ryanodine under these conditions, this should also be apparent from changes in the amplitude of I_{si} during a test pulse following a prepulse to inactivate I_{si}. An experiment to test this possibility is illustrated in Figure 1c. The conditioning prepulse was short (5 ms) so that inactivation would be incomplete. The

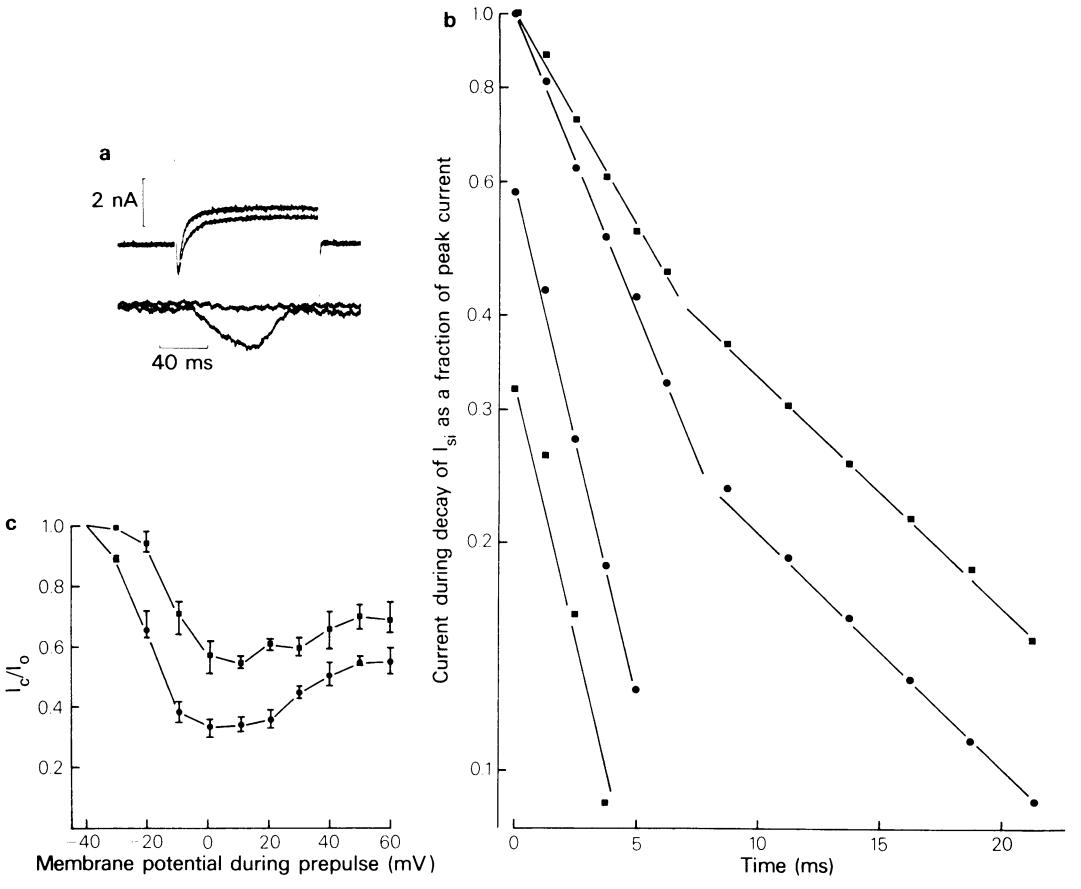

Figure 1 Effects of ryanodine (1 μM) on I_{si} carried by Ca and on contraction. I_{si} and the accompanying contraction were recorded simultaneously, and panel (**a**) shows a pair of traces recorded in this way after 2 min stimulation in ryanodine superimposed on another pair of traces recorded before ryanodine; contraction is shown in the lower two traces, and is indicated by a downward deflection (amplitude in arbitrary units). The records after ryanodine are the slower current and smaller contraction (middle two traces). Panel (**b**) shows the amplitude of I_{si} plotted semilogarithmically against time after peak inward current, before (●) and after (■) prolongation of I_{si}; the fast component of decay in each case is replotted after subtraction of the slow component, extrapolated to 0 ms. The time constants of the fast and slow components were 3.4 and 14 ms. Panel (**c**) shows the fractional reduction (I_c/I_o, see text) in the amplitude of I_{si} following a 5 ms prepulse to partially inactivate I_{si}, plotted against membrane potential during the conditioning prepulse. Points represent the mean of from 2 to 9 currents before (●, three cells) or after (■, four cells) prolongation of I_{si} by ryanodine; bars show range.

fractional reduction in the amplitude of I_{si} following the conditioning prepulse was determined from the ratio of the current evoked by a step depolarization from −40 to 0 mV with a preceding conditioning prepulse (I_c), to that evoked by a similar step without a preceding pulse (I_o). Figure 1c shows I_c/I_o plotted as a function of membrane potential during the conditioning prepulse, which was varied over the range −40 to + 60 mV. It can be seen that, when contraction was suppressed and I_{si} prolonged by ryanodine, the inactivation measured in this way was less at all conditioning potentials than the inactivation determined in the absence of ryanodine. A small change in steady current at the end of the step depolarization was apparent when I_{si} was prolonged by ryanodine (Figure 1a); whether or not this reflects a non-inactivating component of I_{si} remains for further study.

Both the reduction in the amplitude of contraction and the prolongation of I_{si} by ryanodine were dependent on application of the step depolarizations, since if the cells were preincubated with ryanodine for

15–45 min before stimulation, the first of a series of depolarizations evoked a strong contraction, and this was followed by a progressive reduction in the amplitude of contraction and a prolongation of I_{si} similar to that observed when ryanodine was applied during continuous stimulation.

Discussion These experiments show that the second inward current carried by Ca is prolonged while contraction is suppressed by ryanodine in rat ventricular muscle cells. It appears that ryanodine suppresses contraction by an effect on excitation-contraction coupling subsequent to I_{si}. A reduction of contraction without abolition of I_{si} has also been reported for calf Purkinje fibres by Kenyon & Sutko (1983). It has been suggested by Sutko *et al.* (1979) that ryanodine reduces the amount of Ca available for contraction, and morphological evidence has been presented for an action of ryanodine at the junction between transverse tubules and the sarcoplasmic reticulum (Penefsky, 1974). Sutko & Willerson (1980) present evidence that ryanodine inhibits the release of Ca from intracellular stores, noting that the order of sensitivity to ryanodine of different species (rat > dog = cat > rabbit) mimics the relative dependence of these species on intracellular Ca for force development (cf. Fabiato & Fabiato, 1979).

The results presented here are also consistent with the hypothesis that ryanodine treatment reduces the rate of inactivation of I_{si}. Previous studies (Mitchell *et al.*, 1983) have shown that inactivation of I_{si} by long (100 ms) conditioning prepulses is complete at a prepulse potential of +10 mV and decreases as the prepulse potential is increased over the range +20 to +60 mV. This result was interpreted in terms of a dependence of inactivation of I_{si} on internal Ca (cf. Tillotson, 1979; Ashcroft & Stanfield, 1982; Standen & Stanfield, 1982). The ryanodine-induced reduction in the rate of inactivation is consistent with a reduction by ryanodine in the amount of intracellular Ca available for inactivation of I_{si}. Such a reduction might result from a fall in the release of Ca from internal stores, if ryanodine-sensitive release can contribute to the Ca for inactivation. The observation that stimulation of the cell by step depolarizations was required before the effects of ryanodine, both on contraction and inactivation, became apparent requires further study, and may perhaps reflect the progressive development of an action of ryanodine at an intracellular site.

This work was supported by grants from the B.H.F. and M.R.C.

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(Received September 8, 1983.)