Excess noise in modified conductance states following the interaction of ryanoids with cardiac ryanodine receptor channels

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Abstract The interaction of ryanodine with the ryanodine receptor (RyR) produces profound changes in channel function. Open probability increases dramatically and conductance is reduced. In this report we describe differences in the properties of reduced conductance states produced by the interaction of ryanodine derivatives with RyR channels. Some reduced conductance states are considerably noisier than the normal open state of the RyR channel. Inspection and analysis of these events reveals that the excess noise arises from transitions between two conductance states. Following the interaction of certain ryanodine derivatives, RyR channels undergo transitions between two conformations with slightly different ion-handling properties.

Key words: Calcium-release channel; Sarcoplasmic reticulum; Ryanodine receptor; Ryanodine

1. Introduction

Ryanodine receptors (RyRs) are cation-selective membrane ion channels that provide pathways for the regulated release of Ca^{2+} from intracellular storage organelles such as the endoplasmic or sarcoplasmic reticulum [1–3]. This class of channels are termed RyRs because each functional channel possesses a single high affinity binding site for this plant alkaloid.

The specificity of the ryanodine binding site, and the regions and physical properties of the ryanodine molecule that are responsible for its high affinity interactions, have been investigated by monitoring and analysing the binding of natural congeners and synthetic derivatives of ryanodine (ryanoids) to the receptor sites on populations of channels in isolated membrane vesicles [4]. The functional consequence of the interaction of ryanodine with the high affinity binding site on RyR channels in native membrane vesicles is an increase in the Ca^{2+} permeability of the membrane [5]. The mechanisms underlying this increased permeability were revealed when micromolar concentrations ryanodine were applied to individual RyR channels reconstituted into planar phospholipid bilayers. Under these conditions the interaction of ryanodine with RyRs results in marked alterations in function; channel open probability (Po) increases dramatically and channel unitary conductance is reduced [6]. With alkaline, earth divalent cations as the charge carrying species the fractional conductance (FC; single channel conductance of the modified state expressed as a proportion of the conductance in the absence of ryanodine) is less than 0.4, while with the group 1a monovalent cations values of FC are within the range 0.60–0.66 [7]. Altered unitary conductance reflects changes in both the affinity of the conduction pathway of the RyR channel for cations and the relative permeability of cations within the channel. These changes are thought to occur as the result of conformational alterations in the conduction pathway of the channel induced by the binding of ryanodine [7]. The application of ryanoids of varying structure to individual RyR channels has revealed that the FC induced by the binding of these molecules to the channel is governed by structural features of the ligand [8] and comparative molecular field analysis has revealed strong correlations between FC and specific structural loci on the ryanoid molecule [9].

It has been assumed that the interaction of a single ryanoid molecule with the high affinity ryanoid binding site on RyR gives rise to a single modified conductance state. However, detailed inspection of modified conductance states induced by a large number of ryanoids reveals that some of these ryanoid-induced states display excess noise when compared with the normal open-channel conducting state. Such events could result from transitions between more than one conductance state. In this communication we describe examples of such 'noisy' ryanoid-induced states and investigate the mechanisms responsible for their occurrence.

2. Materials and methods

Phosphatidylethanolamine was purchased from Avanti Polar Lipids Inc. and phosphatidylcholine from Sigma-Aldrich. Standard chemicals were obtained as best available grade from BDH Ltd. or Sigma-Aldrich. Ryanodine was supplied by Agrisystems International, and 21-azido-9α-hydroxyryanodine, 21-amino-9α-hydroxyryanodine and 8β-amino-9α-hydroxyryanodine were synthesised as described earlier [9]. Ryanoids were stored as stock solutions in 50% ethanol at −20°C.

Heavy sarcoplasmic reticulum membrane vesicles were prepared from sheep cardiac muscle [10] and RyR channels isolated and reconstituted into unilamellar liposomes [11] as described previously.

Planar phospholipid bilayers were formed from suspensions of phosphatidylethanolamine in *n*-decane and individual RyR channels

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incorporated into the bilayer following previously described methods [12]. Single channel current fluctuations were monitored with K^+ as the charge carrying species and data stored on Digital Audio Tape. Single channel current amplitudes were measured from digitised data [12] and data are quoted as mean values ± S.E.M.

3. Results and discussion

(Fig. 1) shows examples of altered channel function following the interaction of various ryanoids with individual RyR channels. In all cases ryanoid binding results in the occurrence of a high Po, reduced conductance, state. However, inspection of these traces indicates significant differences in channel activity when the ryanoids are bound to the channel. The modified conductance states induced by ryanodine and 21-azido-9α-hydroxyryanodine are 'quiet'; that is the current noise in the ryanoid-modified state is comparable to that of the normal open state of the channel. In contrast, it is clear that the current noise associated with the modified conductance states induced by both 21-amino-9α-hydroxyryanodine and 8β-amino-9α-hydroxyryanodine is significantly greater than that of the normal open state of the channel. With closer inspection it can be seen that with these ryanoids bound, the RyR channel fluctuates between two modified conductance states. Rates of transition between these states are very fast in the case of 21-amino-9α-hydroxyryanodine and as a consequence the individual dwell times in each state are poorly resolved. With 8β-amino-9α-hydroxyryanodine transition rates are slower and dwell times in the two states (α and β) are clearly resolved.

What mechanisms underlie this apparent fluctuation between different conductance states following the interaction

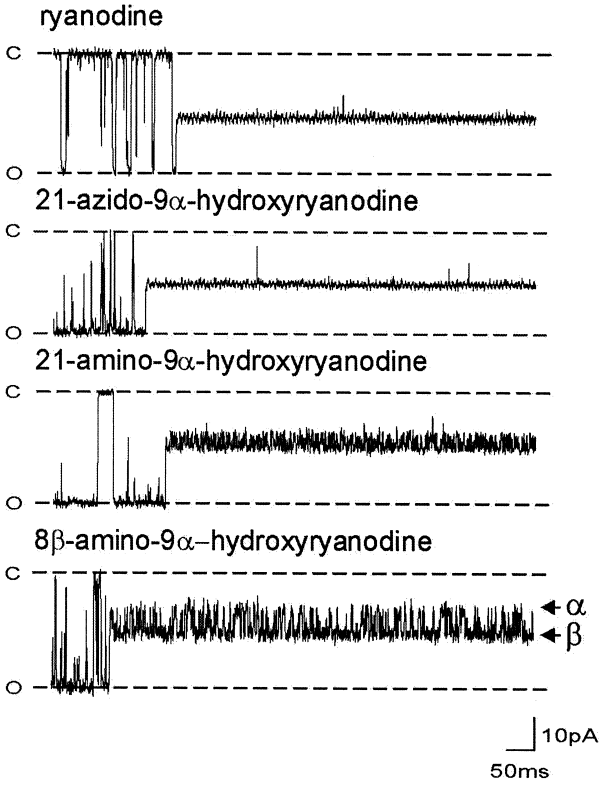

Fig. 1. Modification of function following the interaction of the indicated ryanoids with single RyR channels. In all cases K^+ is the charge carrying species and the holding potential is +40 mV.

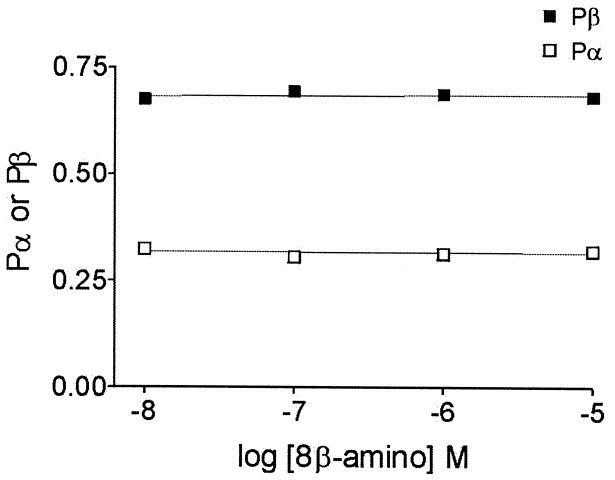

Fig. 2. Relationship between the probability of occurrence of α and β modified conductance states and concentration of 8β-amino-9α-hydroxyryanodine in the solution at the cytosolic face of the RyR channel. Points are mean ± S.E.M., $n = 4–6$. Holding potential is +60 mV.

of some ryanoids with RyR? We consider there to be two possible explanations for the observed behaviour. The first of these is that following the interaction of a ryanoid with the RyR channel, and the subsequent induction of a modified conductance state, unbound ryanoid in the bulk solution enters the conduction pathway of the ryanoid-modified channel and acts as a partial blocker of K^+ translocation through the channel. Both 21-amino-9α-hydroxyryanodine and 8β-amino-9α-hydroxyryanodine have a net positive charge and there are examples in the literature of partial block of RyR channels by large cations including tetraalkylammoniums [13] and local anaesthetics [14]. Under these circumstances the β-conductance state observed in RyR in the presence of 8β-amino-9α-hydroxyryanodine would be the modified conductance state induced by the interaction of the ryanoid with the channel and the α-conductance state would represent a partial blocked state of the ryanoid-modified state. The feasibility of this mechanism can be investigated by simply varying the concentration of 8β-amino-9α-hydroxyryanodine in the solution at the cytosolic face of the channel. If the observed α-subconductance state is a partial blocked state of the ryanoid-modified state, an increase in ryanoid concentration will increase the probability of occurrence of the blocked (α) state whilst decreasing the probability of occurrence of the β state. We have investigated this possibility by monitoring the probabilities of occurrence of the α- and β-conductance states during modified conductance events in the presence of cytosolic 8β-amino-9α-hydroxyryanodine at concentrations ranging from 10 nM to 10 μM at a holding potential of +60 mV. The results of these investigations are shown in Fig. 2. Clearly there is no variation in the probability of occurrence of either the α or β states within a 1000-fold concentration range of the ryanoid. These observations indicate that the α-subconductance state is not a partial blocked state of a single modified conductance state induced by 8β-amino-9α-hydroxyryanodine.

The alternative explanation for the observed behaviour is that with the ryanoid bound, RyR fluctuates between two conductance states (α and β). This in turn suggests that

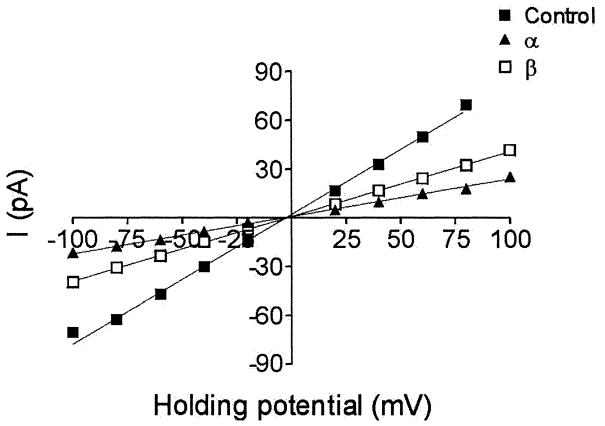

Fig. 3. Variations in unitary current amplitude with holding potential for the full open conductance state (control) and the α and β modified conductance states induced by 8β-amino-9α-hydroxyryanodine. Points are mean ± S.E.M. Control, $n = 6–18$; α, $n = 4–9$; β, $n = 4–9$.

with a ryanoid, such as 8β-amino-9α-hydroxyryanodine occupying the high affinity ryanoid binding site on RyR, the conduction pathway of the channel fluctuates between two conformations with slightly different ion-handling properties [7,15]. Consistent with this proposal, the unitary current amplitudes of both the α and β states vary linearly with holding potential (Fig. 3). The α and β states have values of FC of 0.31 ± 0.01 and 0.56 ± 0.01 respectively ($n = 4–9$).

In previous investigations we have demonstrated that, in addition to modifying rates of permeant ion translocation, the interaction of a ryanoid with the RyR channel alters the effectiveness of tetraethylammonium (TEA^+) as a concentration- and voltage-dependent blocker of K^+ translocation. Broadly, the effectiveness of TEA^+ as a blocker of a ryanoid-modified RyR channel correlates with the FC of the modified state induced by the ryanoid; the smaller the FC induced by a ryanoid, the greater the concentration of TEA^+ that is required to produce block. These ryanoid-induced changes result from alterations in the voltage dependence of the TEA^+ blocking reaction and in some cases the affinity of the conduction pathway for the blocking cation [15].

We have used TEA^+ to investigate the nature of the α and β conductance states induced by 8β-amino-9α-hydroxyryanodine. Fig. 4 shows current fluctuations of a single RyR channel in the presence of 10 μM 8β-amino-9α-hydroxyryanodine at holding potentials ranging from −80 to +80 mV. The left-hand panel shows control traces whilst the right-hand panel shows representative traces from the same channel following the addition of 20 mM TEA^+ to the solutions at both the cytosolic and luminal faces of the channel. Relationships between unitary current amplitude and holding potential for the full open conductance state and the α- and β-conductance states in the absence and presence of 20 mM TEA^+ are shown in Fig. 5 ($n = 4–9$).

In the absence of ryanoids, TEA^+ is a concentration- and voltage-dependent blocker of K^+ translocation when present at the cytosolic face of the RyR channel [15,16]. As can be seen in Fig. 5a, the degree of block produced by TEA^+ increases as trans-membrane voltage is taken to more positive potentials; at a holding potential of 80 mV, 20 mM TEA^+ reduces unitary current amplitude of the full open state of RyR from 69.65 ± 0.86 pA to 25.94 ± 0.73 pA (a reduction of 63%). TEA^+ also blocks K^+ translocation in the α and β conductance states induced by 8β-amino-9α-hydroxyryanodine, however, the degree of block is significantly less than that seen in the full open state. At a holding potential of 80 mV, 20 mM TEA^+ reduces the unitary current amplitude of the α state from 17.77 ± 1.21 pA to 11.13 ± 0.89 pA (a reduc-

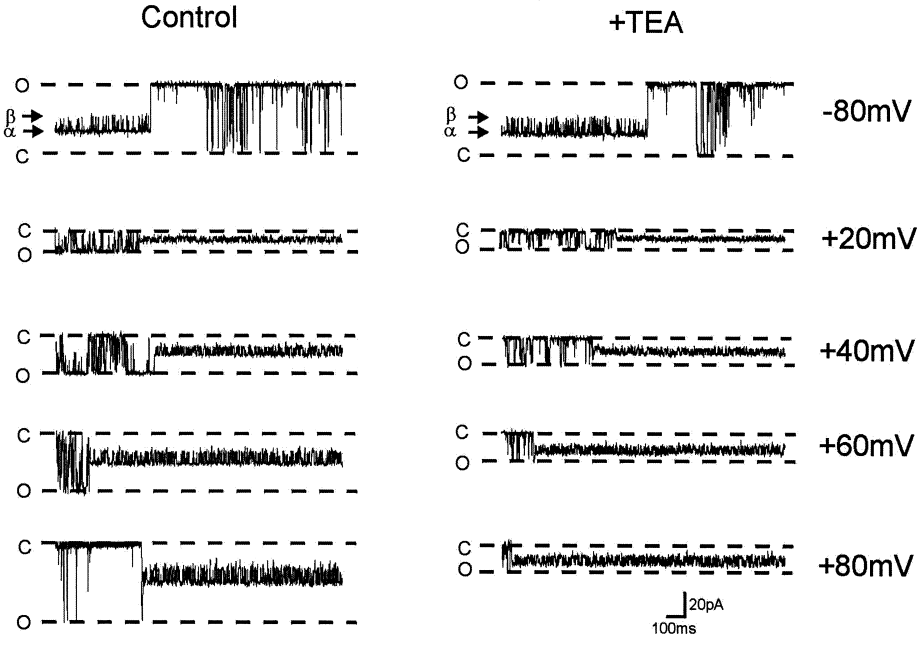

Fig. 4. Block of full open conductance state and the α and β 8β-amino-9α-hydroxyryanodine-induced modified conductance states of a representative channel by symmetrical 20 mM TEA^+. Left panel shows variation in unitary current amplitude at the indicated holding potentials in the absence of TEA^+. The right panel shows equivalent current fluctuations in the presence of TEA^+.

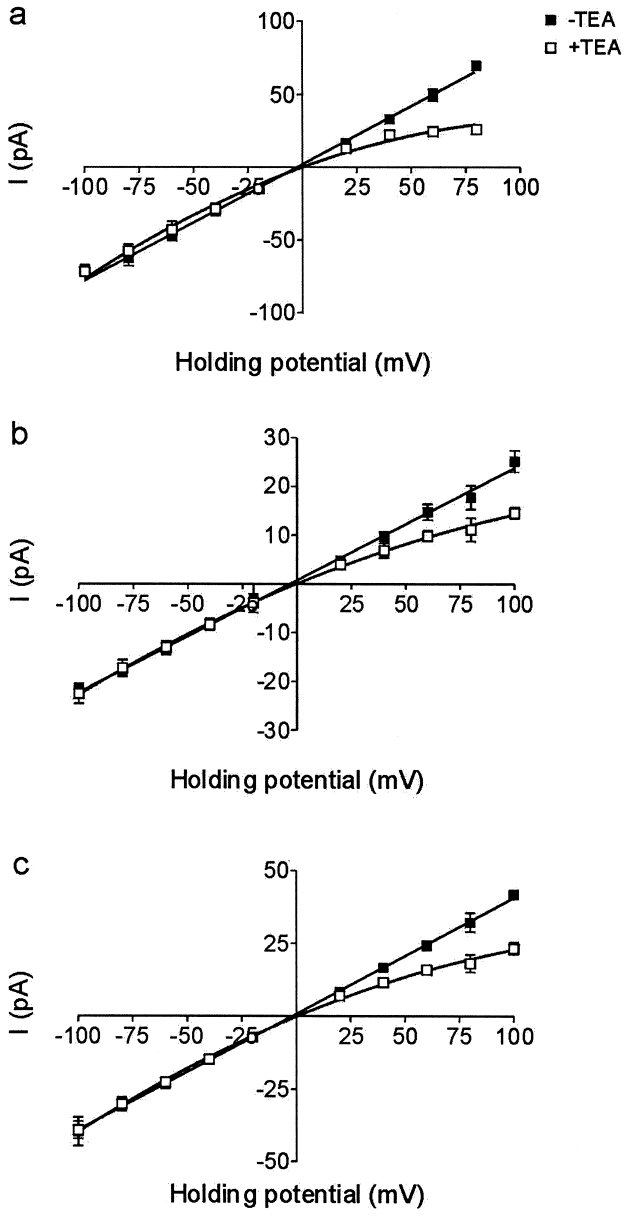

Fig. 5. Current–voltage relationships for the full open conductance state (a) and the α (b) and β (c) 8β-amino-9α-hydroxyryanodine-induced modified conductance states of RyR in the absence (filled symbols) and presence (open symbols) of symmetrical 20 mM TEA^+. Points are mean ± S.E.M. Control ± TEA^+, $n = 6–18$; α−TEA^+, $n = 4–9$; α+TEA^+, $n = 6–8$; β−TEA^+, $n = 4–9$; β+TEA^+, $n = 6–8$.

tion of 37%) (Fig 5b) and that of the β state from 32.24 ± 1.44 pA to 18.13 ± 1.16 pA (a reduction of 44%) (Fig 5c). These data are consistent with the proposal that the α and β states seen in the presence of 8β-amino-9α-hydroxyryanodine are different conductance states reflecting different conformations of the RyR conduction pathway induced by the interaction of this ryanoid with the channel. When compared with its action on the control full open state of the channel, TEA^+ is a significantly less effective blocker of the ryanoid-induced α and β states. The small difference in effectiveness of TEA^+ in the α and β states is entirely consistent with observations made previously with ryanoids that induce single, 'quiet', modified conductance states [15]. Similarly, Woodhull analysis [17] of the data presented in Fig. 5 demonstrates that the reduced effectiveness of TEA^+ in the α and β states induced by 8β-amino-9α-hydroxyryanodine results from alterations in the voltage dependence and affinity of the TEA^+ blocking reaction (values of $z\delta$ and $K_b(0)$ for block of the open, α and β states are respectively: $z\delta = 0.88$, $K_b(0) = 167.3$ mM; $z\delta = 0.45$, $K_b(0) = 139.3$ mM; $z\delta = 0.52$, $K_b(0) = 147.8$ mM). These observations indicate that both the α and β conductance states have properties that characterise them as separate ryanoid-modified states and result from rearrangements in the structure of the conduction pathway of the RyR channel following the binding of 8β-amino-9α-hydroxyryanodine.

We conclude that the interaction of ryanoids with the high affinity binding site on the RyR channel protein induces profound effects on channel function and that the ryanoid-induced alteration in ion handling by the channel is dependent upon the structure of the ligand. In most cases ryanoid interaction produces changes in the conformation of the conduction pathway of the channel that results in altered ion handling and yields a single modified conductance state [7]. Some ryanoids can induce more than one FC state. In the case of 21-*p*-nitrobenzoylamino-9α-hydroxyryanodine we have observed three modified conductance states associated with the interaction of three different conformers of this flexible ryanoid [15]. In each case the modified state is induced by the interaction of a conformer of the ryanoid with the open RyR channel; there is no transition between conductance states whilst the ryanoid is bound. In contrast, the novel data presented in this communication demonstrate that with ryanoids such as 21-amino-9α-hydroxyryanodine and 8β-amino-9α-hydroxyryanodine, binding produces RyR channels that display transitions between two states whilst the ryanoid is bound. Each state possesses properties characteristic of a ryanoid-modified conductance state. With these ryanoids bound, the conduction pathway of the RyR channel can exist in conformations with slightly different ion-handling properties. The factors governing the conductance properties of these states and the rates of transition between the states remain to be established.

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