Adenosine Discriminates between the Caffeine and Adenine Nucleotide Sites on the Sheep Cardiac Sarcoplasmic Reticulum Calcium-Release Channel

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Abstract. Calcium-release channels of sheep cardiac sarcoplasmic reticulum were incorporated into phosphatidylethanolamine bilayers and single channel currents were recorded under voltage-clamp conditions. The effect of adenosine on single channel conductance and gating was investigated, as were the interactions between adenosine and caffeine and adenosine and α,β-methylene ATP.

Addition of adenosine (0.5–5 mM) to the cytosolic but not the luminal side of the membrane increased the open probability of single calcium-activated calcium-release channels by increasing the frequency and duration of open events, yielding an EC_{50} of 0.75 mM at 10 μM activating Ca^{2+}.

Addition of 1 mM caffeine potentiated the effects of adenosine at 10 or 100 μM-activating cytosolic calcium, but had no effect on the inability of adenosine to activate the channel at 80 μM calcium, suggesting discrete sites of action on the calcium-release channel for adenosine and caffeine. In contrast, addition of 100 μM α,β-methylene-ATP decreased single channel open probability in the presence of adenosine, suggesting that these compounds act on the same site on the channel.

Activation of single channel opening by adenosine, or by adenosine together with caffeine, had no effect on single channel conductance or the Ca^{2+}/Tris{+} permeability ratio. Channels activated by adenosine were characteristically modified by ryanodine and blocked by μM ruthenium red or mM magnesium.

These results show that adenosine activates the sheep cardiac sarcoplasmic reticulum Ca^{2+}-release channel by increasing the frequency and duration of open events in a Ca^{2+}-dependent manner. The receptor site on the channel for adenosine is distinct from that for caffeine but probably the same as that for adenine nucleotides.

Key words: Adenosine — Sarcoplasmic reticulum — Cardiac Ca^{2+}-release channel — Caffeine — Adenine nucleotides

Introduction

Calcium-induced calcium-release, where calcium influx during the action potential causes rapid efflux of calcium from the sarcoplasmic reticulum (SR), is thought to be the main mechanism for releasing stored calcium in cardiac muscle cells (Fabiato, 1985). When isolated cardiac SR vesicles are incorporated into planar phospholipid bilayers, a calcium-selective channel is seen which has a conductance of 80–120 pS with Ca^{2+} as the permeant ion (Rousseau et al., 1986; Ashley & Williams, 1990). Single channel, radioisotope flux and 3H-ryanodine binding studies using isolated SR vesicles indicate that activation of the Ca^{2+}-release channel, rapid release of 45Ca^{2+} from and stimulation of 3H-ryanodine binding to populations of these vesicles is, in general, Ca^{2+} dependent and that other ligands may act in conjunction with Ca^{2+} to increase its effect (Rousseau et al., 1986; Meissner & Henderson, 1987; Holmberg & Williams, 1990; Pessah & Zimanyi, 1991). The activation of these processes by Ca^{2+} is enhanced by several positively inotropic compounds including xanthines, ATP and cardiac glycosides (Rousseau et al., 1986; Meissner, 1986; Meissner & Henderson, 1987; McGarry & Williams, 1993a) and inhibited by magnesium, ruthenium red and calmodulin (Meissner &
Henderson, 1987). Single channel experiments, efflux studies and binding assays have also yielded important results about the skeletal Ca\(^{2+}\) release channel and its modulation by drugs such as ATP and caffeine (Palade, 1987; Rousseau et al., 1988; Chu et al., 1990; Zimanyi & Pessah, 1991). However, it would be unwise to conclude that agents acting on the skeletal isoform of the Ca\(^{2+}\) release channel act by a similar mechanism on the cardiac channel, as some drugs, for example, cardiac glycosides, are specific activators of only the cardiac channel (McGarry & Williams, 1993a); also, the different sensitivities of the isoforms of the channel to Ca\(^{2+}\) mean that drugs which act by a Ca\(^{2+}\)-dependent mechanism will have different potencies on cardiac and skeletal channels (Chu et al., 1991). Hence, this study will concentrate on cardiac SR Ca\(^{2+}\) release channels.

Caffeine has been used extensively to induce positive inotropy and to cause calcium release in whole cells from SR vesicles and to activate SR Ca\(^{2+}\) release channels incorporated into lipid bilayers (Chapman & Miller, 1974; Rousseau & Meissner, 1989; Sitsapesan & Williams, 1991). The mechanism of this activation was shown by Sitsapesan and Williams (1991) to involve both a Ca\(^{2+}\)-dependent increase in the frequency and duration of open events and a Ca\(^{2+}\)-independent action, usually requiring higher concentrations of caffeine.

Although caffeine and adenosine are very similar in structure, and are antagonistic at cell surface adenosine receptors (Olsson & Pearson, 1991), the effect of adenosine on cardiac SR Ca\(^{2+}\) release channel gating has not been studied. The site of action of adenosine with respect to other adenine-containing drugs, or the structurally related drug caffeine, on the cardiac channel has also not been distinguished, although Rousseau and Meissner (1989) suggested, from a limited amount of data, that ATP and caffeine may act at different sites on the cardiac Ca\(^{2+}\) release channel.

The binding of adenosine to high affinity cell surface receptors and the subsequent effects elicited are well characterized (Olsson & Pearson, 1991). However, the intracellular effects of adenosine, in contrast to caffeine, are less well known. Adenosine is a weak inhibitor of type III phosphodiesterase and inhibits adenylylate cyclase by binding to the inhibitory, "P," site of the enzyme (Rodbell, 1983). Here, evidence is presented that adenosine, like caffeine, also acts on the SR Ca\(^{2+}\) release channel.

The investigation of the activation mechanism of the Ca\(^{2+}\) release channel by adenosine will firmly establish whether or not adenosine and caffeine act at the same or distinct sites on the cardiac Ca\(^{2+}\) release channel protein. Clarifying this will allow the full characterization of the Ca\(^{2+}\) release channel pharmacology using both adenosine and caffeine analogues. Additionally, this work may indicate whether or not adenosine (an adenine nucleoside) and \(\alpha,\beta\)-methylene ATP (a nucleotide) act at the same or distinct sites on the channel protein, aiding the structure-activity study of the adenosine and/or caffeine site(s).

Materials and Methods

**PREPARATION OF SR MEMBRANE VESICLES**

The methods for the isolation of SR membrane vesicles were as previously described (Sitsapesan & Williams, 1990). Fresh sheep hearts were obtained from the local abattoir and transported to the laboratory in cold modified cardioplegic solution (Tomlin et al., 1986). Left ventricle and septum (approximately 100 g) were stripped of fat and connective tissue prior to homogenization in a solution containing 300 mM sucrose, 20 mM potassium pipervaine-N'-N'-bis-ethanesulfonic acid (PIPES) and 1 mM phenylmethylsulfonyl chloride (PMSF), pH 7.4. The homogenate was centrifuged at 8,000 \( \times \) g, in a Sorvall GSA rotor for 20 min and the pellet was discarded. The supernatant was then centrifuged at 100,000 \( \times \) g, in a Sorvall T647.5 rotor for 40 min. The mixed membrane population sedimented by this step was resuspended in a solution containing (mM): 400 KCl, 0.5 MgCl\(_2\), 0.5 CaCl\(_2\), 0.5 1,2-di(2-aminoethoxy)ethane-N,N,N',N'-tetraacetic acid (EGTA), 25 PIPES, pH 7.0 and 10% sucrose w/v, and subfractionated on discontinuous sucrose-density gradients. The membrane suspension was layered onto identical salt solutions containing 20, 30 and 40% w/v sucrose and sedimented at 100,000 \( \times \) g, for 120 min in a Sorvall AH629 rotor. Heavy SR (HSR) membrane vesicles collecting at the 30–40% interface were collected and diluted into 400 mM KCl and pelleted by centrifugation at 100,000 \( \times \) g, for 40 min in a Sorvall T647.5 rotor before resuspension in a solution containing 400 mM sucrose, 5 mM \( N'\)-2-hydroxyethylpiperazine-\( N'\)-2-sulfonic acid (HEPES) titrated to pH 7.4 with tris(hydroxymethyl)methylamine (Tris). Membrane vesicles were snap-frozen in liquid nitrogen and stored at -80°C.

**PLANAR LIPID BILAYER METHODS**

Lipid bilayers containing phosphatidylethanolamine in decane (30 mg ml\(^{-1}\)) were formed across a 200 \( \mu \)m diameter hole in the partition between two fluid-filled styrene copolymer chambers, referred to as cis (volume 0.5 ml) and trans (1.0 ml). The trans chamber was held at ground and the cis chamber clamped at holding potentials relative to this. Current flow through the bilayer was measured using an operational amplifier as a current-voltage converter (Miller, 1982). Initially both chambers contained (mM): 50 choline chloride, 10 HEPES and 5 CaCl\(_2\), with the pH adjusted to 7.4 with Tris. HSR vesicles were added to the cis chamber and the choline chloride concentration increased to give a 7:1 gradient cis-trans to promote vesicle fusion with the bilayer. Fusion was marked by the appearance of Cl\(^{-}\)-selective channels (Smith, Coronado & Meissner, 1985). SR vesicles incorporated into the bilayer in a fixed orientation such that the cytosolic face of the Ca\(^{2+}\) release channel was directed towards the cis chamber and the luminal face to the trans chamber. Following vesicle fusion, the cis and trans chambers were perfused with solutions allowing resolution of only Ca\(^{2+}\) release channel currents. The cis chamber was perfused with (mM) 250 HEPES, 125 Tris, pH 7.4; and the trans with 250 mM aspartic acid, 10 HEPES, with the pH adjusted to 7.4 with Ca(OH)\(_2\), giving a [Ca\(^{2+}\)] on the trans side of 67 mM. The concentration of Ca\(^{2+}\) in the cis chamber was buffered to the desired level by the addition of CaCl\(_2\) and EGTA. The concentrations of Ca-