Rp-cAMPS has no effect on adenosine A₁ receptors in guinea-pig cardiomyocytes

Abstract  
Rp-cAMPS, a protein kinase A inhibitor, is used in the investigation of the cAMP-dependent systems. A report by Musgrave et al. (11) has suggested that Rp-cAMPS may also act on adenosine receptors. To determine whether this occurs in guinea-pig ventricular myocytes, Rp-cAMPS was applied in the presence and absence of DCPCX, an adenosine A₁ receptor antagonist. The isoprenaline-induced response was significantly decreased by Rp-cAMPS and the effect was not altered by the presence of DCPCX. Therefore Rp-cAMPS has no effect on cell contraction via adenosine A₁ receptors and can reliably be used to investigate cyclic AMP-dependent systems in isolated cardiac myocytes.

Key words  Cardiomyocyte – beta-adrenoceptors – Rp-cAMPS – adenosine receptors – protein kinase A

Introduction

Cyclic-AMP is a ubiquitous intracellular second messenger which exerts a pivotal role in the regulation of cardiac contraction in response to external stimulation. In the normal heart, myocardial contractility is regulated by the stimulatory sympathetic nervous system. The force of contraction is dependent upon adenylate cyclase activity which is dually regulated by β₁- and β₂-adrenoceptors. These couple through a heterotrimeric stimulatory G-protein (Gs) to cause adenylate cyclase activation whilst m₂-cholinoceptors and A₁ adenosine receptors inhibit this response via an inhibitory G-protein (Gi).

Isolated cardiac myocytes are frequently used in order to understand further the mechanisms by which the syndrome of heart failure and ensuing reduced cardiac contraction is induced. When investigating the role of cAMP on a particular pathway it is often important to block the production or effects of cAMP. Rp-cAMPS is a membrane permeable inhibitor of cyclic AMP-dependent protein kinase (PKA) and since its synthesis in 1984 has been used in many applications to investigate cyclic AMP-dependent systems (3, 7, 12, 16).

In 1993 concerns about the properties of Rp-cAMPS were raised by Musgrave et al., who were using this compound to investigate superoxide anion production from human neutrophils (11). It was thought that the mechanism of superoxide formation was via a cyclic AMP-dependent pathway, so that Rp-cAMPS would decrease the effects of adenylate cyclase and thus enhance superoxide production. However, Rp-cAMPS unexpectedly inhibited superoxide production. They
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subsequently found that the inhibitory effect of Rp-cAMPS was blocked by the adenosine antagonist, XAC, and concluded that either Rp-cAMPS or adenosine itself (as a breakdown product) was acting via adenosine receptors in the preparation. Cardiovascular cells also have membrane adenosine receptors. Cardiomyocytes contain adenosine A₁ receptors which mediate the negative dromotropic, chronotropic, inotropic and anti-β-adrenergic actions of adenosine while vascular cells contain adenosine A₂ receptors which cause vasodilation (13). Activation of adenosine A₁ receptors will reduce Gs-mediated cAMP production. It is therefore possible that an inhibitory effect of Rp-cAMPS on the response to an inotrope could be mediated through the adenosine A₁ receptor rather than PKA.

In the present study we investigated the effect of Rp-cAMPS on isolated guinea-pig cardiomyocytes with and without adenosine A₁-blockade using DCPCX. We report that any effects of Rp-cAMPS were secondary to its cAMP blockade and that it can be reliably used in this model.

Materials and methods

Isolation of guinea-pig myocytes

Adult myocytes were enzymatically isolated as previously described using a Langendorff preparation from male Dunkin-Hartley guinea-pigs (8).

Action of Rp-cAMPS

The cardiomyocytes were constantly superfused and oxygenated (95 % O₂ / 5 % CO₂) with Krebs Henseleit (KH) solution (mmol/L; NaCl 119, CaCl₂ 1.0, KCl 4.7, MgSO₄ 0.94, KH₂PO₄ 1.2, NaHCO₃ 25 and glucose 11.5 at pH 7.4) at a rate of 2 ml/min at 32 °C and electrically stimulated with a biphasic pulse (0.5 Hz, 1 ms, 50 % above threshold) through platinum electrodes placed either side of the bath. A video camera/edge detection system was used to monitor cell contraction as previously described (8). The myocytes were either pre-incubated in KH alone or with KH plus 300 nmol/L DCPCX for 20 min. The contractile response to an increasing concentration of isoprenaline was established and then the solution was recirculated with the maximum concentration of isoprenaline tolerated. Each subsequent concentration was added after steady state was reached which was approximately 5 min. Rp-cAMPS (100 µmol/L final concentration) was added to the reservoir and allowed to recirculate for 40 min. The cells were then reperfused with KH alone to remove the Rp-cAMPS until the contraction amplitude had reached a steady state. The cells were then rechallenged with the maximum dose of isoprenaline for 5 min to confirm that any decrease in contraction amplitude was a result of the action of Rp-cAMPS and not on decreasing cell viability. Adenosine (10 µmol/L) was added to maximum Iso for 15 min to confirm complete adenosine receptor blockade by DCPCX. All drugs were then removed and the cell superfused with KH solution. The total time for each experiment was up to 2 hours 15 min.

Action of adenosine

To confirm the effect of adenosine on contraction of isolated guinea-pig cardiomycytes stimulated by isoprenaline, adenosine (10 µmol/L) was added to a maximally tolerated dose of isoprenaline for 15 min. To confirm any reduction in contraction was a direct result of the adenosine and not due to decreasing cell viability, the cells were rechallenged with the same dose of isoprenaline.

Statistical analysis

Values are stated as mean ± SEM unless otherwise stated. Each cell was from a separate animal preparation unless stated. Statistical comparisons between the myocytes treated with and without DCPCX were analysed using an unpaired t-test. Where the effect of DCPCX on maximum contraction amplitude was assessed, experiments were done on a single myocyte and analysed using a paired t-test. The effects of ISO ± Rp-cAMPS was also compared using a paired t-test.

Results

Intact ventricular cardiomyocytes were tolerant to the Rp-cAMPS compound in micromolar concentrations. No deleterious effects such as hypercontracture, rounding or arrhythmia were observed during its addition or incubation: this is also true for mouse, dog and human myocytes (data not shown). We have previously demonstrated that 100 µmol/L Rp-cAMPS does not affect basal contraction amplitudes of guinea-pig myocytes at any frequency of stimulation (10).

The effects of Rp-cAMPS on maximal isoprenaline response

The effect of Rp-cAMPS (100 µmol/L) on maximal response to isoprenaline is shown in Fig. 1 (n = 6). The response to an increasing concentration of isoprenaline was established and the maximal concentration used thereafter (3 x 10⁻⁹ or 1 x 10⁻⁸ mol/L). Rp-cAMPS decreased the contraction ampli-