Specific activation of adenylyl cyclase V by a purinergic agonist

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Abstract The present study was designed to investigate whether and how the purinergic stimulation of rat ventricular myocytes modulates the cAMP-dependent pathway. Stimulation of cardiomyocytes with ATPγS in the presence of the phosphodiesterase inhibitor IBMX increases by 3-fold intracellular cAMP content. In contrast to β-adrenergic stimulation, the purinergic stimulation of adenylyl cyclase was not inhibited by activation or enhanced by inhibition of a G_i protein. Forskolin did not potentiate the effect of extracellular ATPγS on intracellular cAMP content but the effect of isoproterenol did. Like isoproterenol, the purinergic agonist decreased subsequent ADP-ribosylation of a 45 kDa $G_{\alpha s}$ by cholera toxin. ATPγS also increased cAMP content in neonatal rat cardiomyocytes, a cell type that expresses a long form of G_s protein (α_s, 52 kDa) in contrast to adult rat cardiomyocytes that express mostly a short form of G_s protein (α_s, 45 kDa). Both purinergic and β-adrenergic agonists increased cAMP in HEK 293 cells expressing type V adenylyl cyclase while cAMP was only increased by β-adrenergic stimulation of HEK expressing type IV or VI adenylyl cyclases. Thus, we propose that the purinergic and β-adrenergic stimulations differentially activate adenylyl cyclase isoforms in rat cardiomyocytes and that adenylyl cyclase V is the specific target of the purinergic stimulation.

Key words: ATP; Adenylyl cyclase; Cardiomyocyte; Heart failure

1. Introduction

Extracellular purines have been recognized for many years to exert various and potent actions on the cardiovascular system (for review, [1,2]). These actions are mediated by different subtypes of purinergic receptors, namely the P_1-purinoceptor with high affinity for adenosine and the P_2-receptor that is preferentially activated by ATP and ADP. Furthermore, P_2-purinoceptors can be classified into different subtypes, such as P_{2X}, P_{2Y}, P_{2Z}, P_{2T} and P_{2U} [3]. The pharmacological and molecular classifications of these latter is still under debate while the cloning of numerous P_{2X} and P_{2Y} is under progress [3]. A least four P_{2X} (i.e. P_{2X1}, P_{2X3}, P_{2X4}, P_{2X5}) and four P_{2Y} (i.e. P_{2Y1}, P_{2Y2}, P_{2Y4}, P_{2Y6}) receptors were already found in heart [4] suggesting that purines exert a major regulation of cardiac function.

ATP, released from nerve terminals under physiological situations or from cardiomyocytes, endothelial cells, smooth muscle cells and platelets under pathological conditions have multiple cellular effects. In the heart, the purine increases contractility of isolated cardiac preparations [5]. It induces a transient acceleration and then slows down the sinus pacemaker leading to a negative chronotropic and dromotropic effect on the mammalian sinoatrial node. In ischemic hearts, ATP could also be a source of arrhythmia [2]. To better understand the mechanism of action of the purinergic agonist on cardiac function, the dissection of intracellular signaling pathways activated by ATP is of major interest.

Extracellular ATP triggers the breakdown of phosphoinositides in most cell types [6] including cardiomyocytes leading to IP_3 formation [5,7] and PKC activation [8]. The purine also activates a tyrosine kinase-dependent pathway [9]. In some cell types, including hepatocytes [10], platelets [11] and glioma cells [12], ATP decreases intracellular cAMP through activation of a pertussis toxin-sensitive G protein while in others such as endothelial cells [13] or neuronal NG108 cells [14] the purine is a potent activator of adenylyl cyclase leading to an increase in intracellular cAMP. Whether ATP does affect intracellular cAMP levels in cardiac cells is still controversial. Zheng et al. [15] observed that ATP does not affect basal cAMP content of rat ventricular cardiomyocytes but further increases the β-adrenergic receptor-mediated cAMP rise. On the contrary, Yamada et al. [7] used cardiomyocytes isolated from fetal mice and found a decrease in isoproterenol-induced cAMP increase by ATP but no significant effect of the purine when applied alone to the cells. We thus decided to reconsider the question as to whether ATP modulates the cAMP-dependent pathway in rat cardiomyocytes. In the present study, we have used different approaches to modulate basal content of intracellular cAMP to further investigate the effect of P_2-purinergic stimulation on cyclic nucleotide levels in isolated cardiomyocytes. We found that purinergic stimulation of cardiomyocytes does significantly increase intracellular cAMP. This effect was additive to increases induced by isoproterenol but was not counteracted by G_i protein stimulation. Purinergic and β-adrenergic stimulations of transiently transfected HEK 293 cells that express specific adenylyl cyclase isoforms showed that adenylyl cyclase type V is specifically activated by the purine. This concept as to a specific targeting of an adenylyl cyclase isoform that we found in both cardiac cells and in the HEK 293 cell line could be extended to other cell types that express purinergic receptors.

2. Materials and methods

2.1. Cardiomyocyte isolation

Cardiomyocytes were isolated from hearts of 200–250 g male Wistar rats as previously described [16]. Briefly, the hearts were first perfused at 35°C with a nominally Ca^{2+}-free HEPES-buffered solution for 5 min and then for 55 min with the same solution containing 1.2–1.5 mg/ml collagenase (Type A, Boehringer) and 20–25 μM Ca adjusted with a Ca^{2+} ion-selective electrode. The heart was removed from the perfusion set-up and gently dissociated through the bore of a large tip pipette. After filtration, the cells were allowed to decant and the pellet was resuspended in collagenase-free HEPES solution. The cells were incubated for 15 min at 37°C. Meanwhile Ca^{2+} concentration was increased stepwise up to 0.3 mM. Cells were then washed and

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resuspended in HEPES solution containing 1 mM Ca and 0.25% BSA. Six to eight 10^6 rod-shaped cells/heart were routinely obtained. Dissociation giving a low yield of viable cells were discarded.

2.2. Culture of neonatal rat cardiomyocytes

Myocytes were isolated from the ventricles of 2–3 days old rat hearts using 0.4 mg/ml collagenase (Worthington type II) and 0.6 mg/ml pancreatin according to Iwaki et al. [17] in HEPES-buffered solution containing 116 mM NaCl, 20 mM HEPES, 0.8 mM Na_2HPO_4, 5.4 mM KCl, 0.8 mM $MgSO_4$, 5.6 mM glucose, adjusted at pH 7.35 at room temperature. The cardiomyocytes were purified on a Percoll gradient and plated in maintenance medium (DMEM/M199 (4:1 v/v) containing 100 U/ml penicillin and streptomycin) supplemented with 10% horse serum and 5% fetal calf serum. Cells were serum starved overnight before agonist stimulation.

2.3. Transfection of HEK 293 cells

HEK 293 cells were cultured in DMEM supplemented with 10% fetal calf serum. The cells plated in 35 mm dishes were transfected with 10 μg/ml of DOTAP and 2 μg of plasmids encoding type IV, V or VI adenylyl cyclases. Type IV was subcloned in pBFP cDNA vector (Clontech) using the *Eco*RI and *Not*HI restriction sites. After 48 h, the cells were serum starved for 1 h before stimulation with agonists.

2.4. Measurement of intracellular cAMP

Cells were preincubated for 10 min in the presence of 0.1 mM IBMX. Batches of 250 000 cardiac cells or dishes of HEK 293 (50% confluency) were incubated for 5 min at 37°C with 100 μM ATPγS, 1 μM isoproterenol, and/or with 10 μM forskolin. Incubations were stopped by addition of perchloric acid. After centrifugation, the pellet was dissolved in NaOH and used to estimate protein content according to Bradford [18]. The supernatant was neutralized by K_2CO_3 and the perchlorate precipitate was spun down. An aliquot of the supernatant was used for cAMP assay. A high specific binding protein assay kit (Amersham) was used to determine cAMP content. Results were expressed as pmoles cAMP/mg cell protein.

2.5. Pertussis toxin treatment of cells

Isolated adult rat cardiomyocytes were incubated for 4 h with 300 ng/ml of pertussis toxin. Such a treatment was previously shown to inhibit by 75% subsequent ADP-ribosylation by the toxin [19]. Some experiments were also performed using cells incubated for 20 h at 37°C in the presence of 100 ng/ml of pertussis toxin. Under the latter condition, 100% of subsequent ADP-ribosylation was inhibited [19]. For such a purpose, cells were suspended in serum-free culture medium (DMEM/M199 4:1 v/v) and were plated at a density of 3×10^5 cells/60 mm plastic dishes and allowed to attach to the plastic for 1 h. The dishes were washed twice and fresh medium containing the toxin was added. Cells were kept overnight under 10% CO_2.

2.6. ADP-ribosylation of G_s

Cardiac crude membranes were prepared from isolated adult and 3 days cultured neonatal cells as already described [20]. ADP-ribosylation of G_s protein was carried out according to Hilal-Dandan et al. [19] with preactivated cholera toxin.

2.7. SDS-PAGE and Western blotting of G_s protein

Cardiac membrane proteins (100 μg) were loaded on 10% acrylamide gel and run overnight according to Laemmli [21]. The gels were stained, destained, dried and ^{32}P content was analyzed by autoradiography on NEN films. Bands on films were quantified using an imaging system (SCION IMAGE). Western blotting was performed as already described [16] using an anti-α_s antiserum.

2.8. Statistics

Statistical significance was assessed as appropriate by a Student's *t*-test with a significant difference as being established at $P \leq 0.05$.

3. Results

3.1. Purinergic stimulation of cardiac cells increases intracellular cAMP content

The first part of this study was aimed at investigating the effect of a purinergic stimulation of cardiomyocytes on basal intracellular cAMP content. Basal cAMP content of isolated rat cardiomyocytes incubated for 10 min at 37°C in the presence of 100 μM IBMX was 12 ± 2 pmoles/mg protein ($n = 38$ out of 19 heart experiments). Purinergic stimulation of cells for 5 min by 100 μM ATPγS, a poorly hydrolysable analog of ATP increased by 3-fold intracellular cAMP content (Fig.

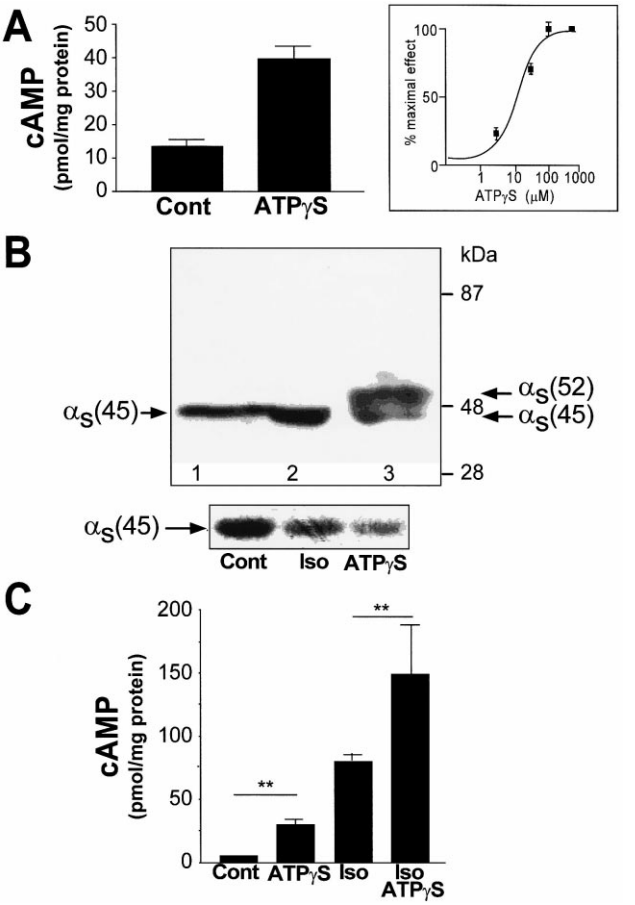

Fig. 1. Effects of extracellular ATP on intracellular cAMP content of isolated cardiomyocytes is mediated by a G_s protein. Adult rat cardiomyocytes were incubated for 10 min in the presence of IBMX and then stimulated for 5 min with 100 μM ATPγS. A: The graph shows the mean ± S.E.M. of 19 experiments performed in duplicate. The inset shows a dose response curve (mean ± S.E.M. of three experiments). B: Top panel: 200 μg of membrane protein from adult cardiomyocytes (lane 1) or 4 μg of purified recombinant α_{s45} (lane 2) or 150 μg of membrane protein from neonatal cardiomyocytes maintained in culture for 3 days (lane 3) were run in SDS-PAGE. The Western blots were probed with an anti-α_s antiserum and the G protein was revealed using ECL detection. Molecular mass standards are shown on the right. Bottom panel: membrane proteins prepared from adult rat cardiomyocytes not stimulated (lane 1), stimulated for 1 min with 1 μM isoproterenol (lane 2), or 100 μM ATPγS (lane 3) were treated with cholera toxin and [^{32}P]NAD. Proteins were then run in SDS-PAGE; the gel was dried and exposed for 24 h to autoradiography films. The arrow indicates the 45 kDa α_s subunit. Quantitation of the number of pixels per band after background substraction was 7231, 4059 and 3082 for lanes 1 to 3, respectively. Similar results were obtained in two other experiments. C: Effects of ATPγS and isoproterenol on intracellular cAMP content of neonatal rat cardiomyocytes. Neonatal cells maintained in culture for 3 days were serum starved overnight and stimulated with 100 μM ATPγS or 1 μM isoproterenol or both in the presence of IBMX. The experiment was performed in three different cultures with similar results. Statistically significant, $*P \leq 0.05$; $**P \leq 0.01$.

1A). Cellular cAMP content went up to 38.0 ± 5 pmoles/mg protein ($n = 38$ out of 19 experiments). No further increase was observed when cells were stimulated for 15 min with the agonist. The EC_{50} for the effect was 15 μM and 100 μM of the agonist was required to obtain a maximal stimulation (inset of Fig. 1A). The same purinergic effect was observed when milrinone (3 μM) together with RO-20-1724 (10 μM) replaced IBMX to inhibit phosphodiesterases (4 ± 0.6-fold stimulation, $n = 3$).

3.2. ATPγS-induced rise in intracellular cAMP is mediated by a G_s protein

Preliminary experiments using pharmacological kinase inhibitors or intracellular Ca^{2+} chelators have excluded the involvement of a protein kinase (e.g. protein kinase C, tyrosine kinase or a Ca^{2+}-dependent kinase) in the purinergic increase in cAMP. The use of indomethacin as an inhibitor of cyclooxygenase and in turn of prostaglandin formation ruled out the possibility that ATPγS increases cAMP in cardiac cells through a autocrine/paracrine effect as previously shown in MDCK cells [22]. Thus, two experimental approaches were used to specifically investigate whether ATPγS could exert its enhancing effect on intracellular cAMP content by directly activating a G_s protein.

We first performed experiments designed to identify the G_s protein switched on by both the purinergic and the β-adrenergic stimulations. Both a 45 and a 52 kDa $G_{\alpha s}$ protein are expressed in cardiomyocytes. The 45 kDa protein would be the major isoform in adult rat heart [23,24] although this is still controversial (see [25]). The 52 kDa protein would be expressed in a higher amount in neonatal rat hearts [24]. Using an anti-$G_{\alpha s}$ antiserum, Western blot analysis of membrane proteins prepared from isolated adult rat cardiomyocytes showed that the 45 kDa $G_{\alpha s}$ isoform is predominantly expressed in adult cardiomyocytes while the 52 kDa protein is the major isoform in neonatal rat cardiomyocytes (Fig. 1B). Stimulation of adult rat cardiomyocytes for 5 min with 100 μM ATPγS decreased by 49 ± 10% ($n = 3$) the subsequent ADP-ribosylation by cholera toxin. Cell stimulation with 1 μM isoproterenol decreased by 52 ± 7% ($n = 3$) ADP-ribosylation of α_s (Fig. 1B). This effect was not increased by additive purinergic stimulation (54 and 56% decrease in two separate experiments). As an alternative approach to find out the G_s protein involved in the purinergic induction of cAMP formation, we used neonatal cells as a cell type that expresses mainly the 52 kDa to look at the effect of ATPγS on intracellular cAMP values. In this cell model, the purinergic stimulation also increased cAMP. The purinergic effect was additive to the isoproterenol enhancing effect on intracellular cAMP (Fig. 1C). These findings suggest that both the $G_{\alpha s45}$ and the $G_{\alpha s52}$ proteins mediate purinergic activation of adenylyl cyclase.

3.3. β-adrenergic and purinergic agonists use different pathways to rise the cAMP content of cardiomyocytes

The increase in cAMP induced by a maximal concentration of ATPγS was additive to isoproterenol-triggered rise in cAMP (see Fig. 1C). To further investigate this point, cells were stimulated with forskolin to fully activate the adenylyl cyclase, in the presence or absence of the agonists, ATPγS or isoproterenol. When added separately to cell suspensions, the purine and forskolin, both used at maximal concentrations,

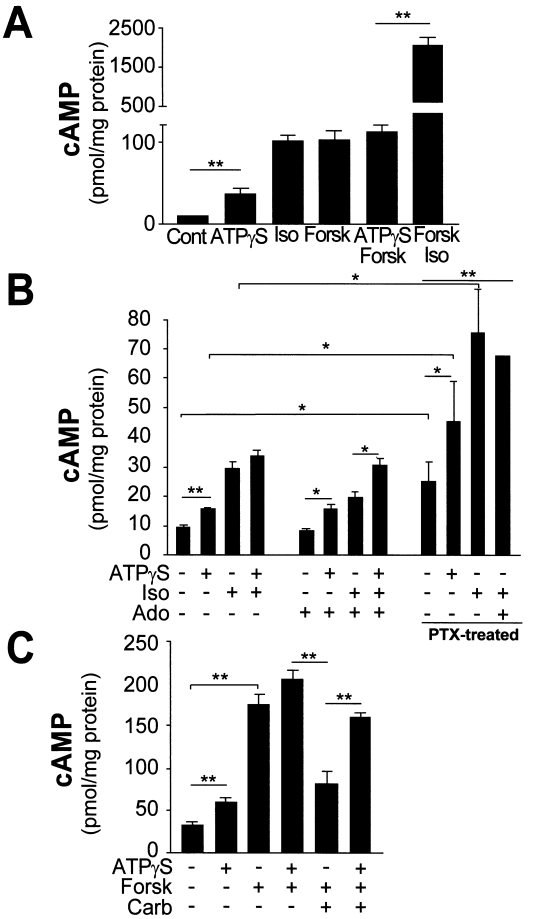

Fig. 2. ATP and isoproterenol use different pathways to increase intracellular cAMP content. A: Synergistic effect of forskolin on isoproterenol- but not on ATP-induced cAMP increase. Adult rat cardiomyocytes were incubated for 10 min in the presence of IBMX and then stimulated for 5 min with 100 μM ATPγS, 1 μM isoproterenol in the presence or absence of forskolin (Forsk, 10 μM). B: Control cells or cells treated for 20 h with 100 ng pertussis toxin were incubated for 5 min with IBMX in the presence or absence of 100 μM adenosine and then stimulated for 5 min with 100 μM ATPγS or/and 10 nM isoproterenol in the sustained presence or absence of adenosine. C: IBMX-treated cells were incubated for 5 min in the absence or presence of 100 μM carbachol and were then stimulated for 5 min with 100 μM ATPγS or/and 10 μM forskolin in the sustained presence or absence of carbachol. Results are duplicates from three cell preparations. Statistically significant, $*P \leq 0.05$; $**P \leq 0.01$.

increased by 3- and 10-fold, respectively, intracellular cAMP content. When ATPγS and forskolin were added together, intracellular cAMP was not further enhanced. On the contrary, β-adrenergic stimulation of cells with 1 μM isoproterenol in the presence of 10 μM forskolin increased by 1000-fold intracellular cAMP values (Fig. 2A). The same effect was observed in the absence of IBMX (data not shown).

3.4. ATPγS-induced rise in intracellular cAMP is not sensitive to a G_i protein

We next considered the possibility that the purinergic stimulation blocks the activity of an inhibitory G_i protein. To test this hypothesis, cells were incubated for 10 min with 100 μM adenosine to switch on G_i protein activity and then stimulated for 5 min in the sustained presence of adenosine with 10 nM

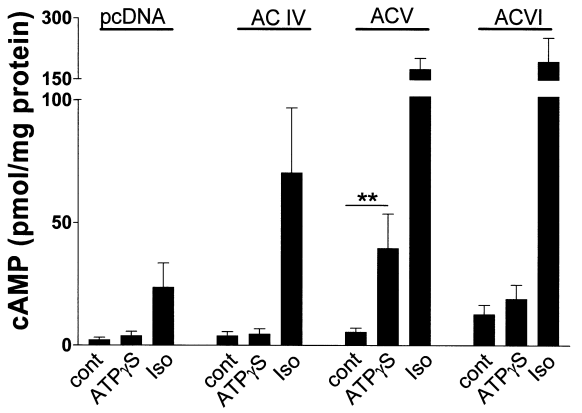

Fig. 3. Effects of ATPγS and isoproterenol on intracellular cAMP of HEK cells overexpressing specific adenylyl cyclase isoforms. HEK 293 cells were transfected with plasmids encoding type IV, V or VI adenylyl cyclase or with empty pcDNA vectors. Cells were then stimulated 48 h later with either ATPγS (100 μM) or isoproterenol (1 μM) in the presence of 100 μM IBMX. Statistically significant, $^{**}P \leq 0.01$.

isoproterenol or 100 μM ATPγS. Adenosine decreased basal cAMP content that suggests that a G_i protein is slightly but sustainedly activated in cardiomyocytes as already observed [25]. Adenosine decreased by 2-fold the isoproterenol enhancing effect on intracellular cAMP values but it did not significantly affect the stimulatory effect of ATPγS (Fig. 2B). Furthermore, ATPγS relieved the adenosine inhibition of isoproterenol effect. As another approach to test the putative involvement of a G_i protein in the effect of ATPγS on intracellular cAMP content, cells were treated with pertussis toxin (PTX, 300 ng/ml for 4 h or 100 ng/ml for 20 h) at 37°C. Such a treatment increased basal intracellular cAMP value (26 ± 5 vs. 10 ± 1 pmol/mg protein, $n = 6$), which confirms the sustained activation of a G_i protein under control conditions. In PTX-treated cells, ATPγS was as much efficient as in non-treated cells to increase intracellular cAMP content (Fig. 2B). However, in PTX-treated cells, adenosine still slightly decreased the isoproterenol effect on intracellular cAMP content although this inhibitory effect was less pronounced than in non-treated cells (compare Fig. 2B middle and right panels). The efficiency of the PTX treatment of cells was further checked by stimulating both non-treated and PTX-treated cells with isoproterenol in the absence or in the presence of the muscarinic agonist carbachol which also activates the G_i protein. Carbachol abolished isoproterenol effect in control cells but not in PTX-treated cells (data not shown). This finding confirms that the PTX-treatment of cells was suitable to inhibit G_i protein activity. Although ATPγS was quite specific of P_2-purinoceptors, it is still possible that the effect of ATPγS on adenosine-treated cells resulted from a displacement of adenosine from its receptor. Thus, similar experiments were carried out using the muscarinic agonist, carbachol, as an activator of the G_i protein. Like adenosine, the muscarinic agonist did not inhibit ATPγS effect on cAMP (data not shown). As expected, carbachol inhibited forskolin-induced cAMP increase in cardiomyocytes. However, ATPγS partially relieved this inhibition (Fig. 2C).

3.5. ATPγS specifically stimulates adenylyl cyclase V

Our data strongly suggested that ATP activates an adenylyl cyclase isoform that is not inhibited by a G_i protein and that did not display a synergism between forskolin and α_s. To identify the adenylyl cyclase(s) isoform(s) that is (are) turned on by either the purinergic or the β-adrenergic stimulation, we used transiently transfected HEK 293 cells. These cells share at least two P_{2Y} purinergic receptors (P_{2Y1} and P_{2Y2}) with cardiomyocytes [26,4], and express $G_{\alpha s45}$ and $G_{\alpha s52}$, but a low level of endogenous type I, II, III, VI and VIII adenylyl cyclase activities [27]. They were thus transfected with plasmids encoding adenylyl cyclase type IV, V and VI adenylyl cyclases, the three isoforms expressed in cardiomyocytes [28]. In pcDNA (empty vector) transfected cells, the purinergic agonist did not affect intracellular cAMP content while isoproterenol increased it by 5-fold. In cells transfected with plasmids encoding type IV or VI, isoproterenol increased by 30–60-fold intracellular cAMP confirming the expression of the cyclases while ATP had no effect on the intracellular cyclic nucleotide level. By contrast, in cells expressing type V adenylyl cyclase, the purinergic stimulation increased by 5–10-fold and isoproterenol by 60-fold intracellular cAMP (Fig. 3).

4. Discussion

ATP is coreleased with noradrenaline from sympathetic nerves. It is thus of physiological relevance to establish whether the purinergic agonist could modulate the same transduction pathway as the one of the β-adrenergic agonist, namely the formation of cAMP. We found that ATPγS used as a specific P_2-purinergic agonist significantly increases intracellular cAMP content of isolated cardiomyocytes. The enhancing effect of the purine on intracellular cAMP is observed when cells are treated with IBMX, or milrinone and RO-20-1724, three potent inhibitors of all phosphodiesterase isoforms. Thus, ATPγS does not exert its action through inhibition of phosphodiesterases but rather stimulates adenylyl cyclase activity. The purinergic effect is additive to the β-adrenergic effect on intracellular cAMP content. These results suggest that the purinergic receptor does not share the same pathway as the one that couples the β-adrenergic receptor to adenylyl cyclase raising the question as to purinergic and β-adrenergic stimulations differentiate at the level of the G protein or of the adenylyl cyclase.

ADP-ribosylation by cholera toxin of G_s protein from membrane prepared from ATPγS-stimulated cells shows that like β-adrenergic receptor, the purinergic receptor is somehow coupled to the 45 kDa α_s subunit. Purinergic and adrenergic effects on subsequent ADP-ribosylation of α_s by the toxin are not additive indicating that both neurohormonal stimulations share the same pool of G_s proteins. Although neonatal cardiomyocytes predominantly feature the expression of 52 kDa $G_{\alpha s}$ subunit, the purinergic agonist also increases intracellular cAMP in the cells. Thus, the 45 kDa protein is not specifically required by the purinergic receptor to activate adenylyl cyclase(s).

Pertussis toxin treatment of cells slightly increases basal cAMP content but does not prevent ATPγS enhancing effect on intracellular cAMP. Thus, a relief of G_i protein inhibitory tone does not mediate the purinergic effect on intracellular cAMP level. The data rather further point to a requirement of a G_s protein in the purinergic transduction pathway that generates cAMP. In contrast to isoproterenol, the effect of ATPγS on intracellular cAMP content is not counteracted

by adenosine or muscarinic stimulation of myocytes. At first glance, this observation suggests that the adenylyl cyclase isoform that is activated by purinergic stimulation is not sensitive to G_i protein and is thus different from the one activated by the β-adrenergic stimulation. Such a hypothesis is strengthened by the observation that purinergic stimulation of cardiomyocytes relieves adenosine- and carbachol-mediated inhibition of isoproterenol or forskolin effect on intracellular cAMP. Although type II adenylyl cyclase is not sensitive in vitro to G_i protein inhibition [29] it is unlikely to be the isoform activated by the purinergic stimulation; this isoform is poorly if not at all expressed in cardiomyocytes [28,29]. In addition, type II adenylyl cyclase is stimulated by PKC, a stimulation that could not be observed in cardiomyocytes (data not shown, [30]). Rather, type V adenylyl cyclase comes out as a better candidate for two reasons. First, this isoform is inhibited in vitro by G_i protein to a lesser extent and for a greater G_i protein concentration than type VI [31]. Second, G_i protein-induced inhibition of type V is dramatically reduced in vitro when the cyclase is activated by G_s [31], a situation similar to the physiological β-adrenergic stimulation (see Fig. 2).

Forskolin exerts a synergistic effect on isoproterenol-mediated increase in cAMP. This confirms the data reported by Fischmeister and Schrier [32] in frog cardiomyocytes and more recently by Post et al. [30] in rat cardiomyocytes. Such an effect can be explained by an enhancement in α_s subunits affinity for adenylyl cyclase that has already bound forskolin. We demonstrate that forskolin does not have the ability to potentiate ATPγS effect on intracellular cAMP. Because only type II, V and VI adenylyl cyclases display enhanced α_s binding by forskolin [33], it further brings evidence that type V and VI are the most predominant isoforms in cardiomyocytes and that the cyclase activity of one of them is turned on by the purinergic stimulus [30]. If the purinergic receptor targets specifically type V adenylyl cyclase, the limited availability of this isoform could also explain the weaker effect of purinergic stimulation on intracellular cAMP content when compared to the β-adrenergic stimulation and the absence of forskolin potentiation on purinergic effect.

Using HEK 293 cells that share at least two P_{2Y} subtypes of receptors with cardiomyocytes [4,26] to express cardiac adenylyl cyclase isoforms, we found that the type V but not IV or VI of adenylyl cyclase is responsive to the purinergic stimulation while all three adenylyl cyclase isoforms are activated by the β-adrenergic stimulation. A lower number of purinergic receptors than of β-adrenergic receptors in HEK cells might account for the weaker effect of ATPγS than the one of isoproterenol on cAMP in adenylyl cyclase V transfected cells. We thus propose that the purinergic receptor like the EGF receptor [34] is specifically coupled through a G_s protein to type V adenylyl cyclase in cardiac cells. This statement also argues in favor of a poor G_i sensitivity of this isoform in cardiac cells in vivo.

We thus have demonstrated that purinergic stimulation of cardiomyocytes increases intracellular cAMP content through a G_s-mediated activation of an adenylyl cyclase isoform different from the one involved in the β-adrenergic stimulation. Our findings open an interesting issue namely the coupling of a G_s protein isoform to a specific adenylyl cyclase isoform. It remains to be determined how stimulation of G_s proteins through a receptor targets a specific isoform of adenylyl cyclase. A specific intracellular localization of adenylyl cyclase isoforms may account for such a targeting. Specific anti-cyclase isoform antibodies are required to test this hypothesis. Differential activation of adenylyl cyclase isoforms by G_s proteins (e.g. different interaction sites G_s/cyclase) may also explain a specific activation of a cyclase isoform by an agonist [34–36]. Furthermore, we report that the purinergic activation of adenylyl cyclase adds to the β-adrenergic effect. That observation suggests that the purine might be a modulator of cell function already regulated by other neuromediators released from the same nerve terminals. Purine-induced increase in intracellular cAMP might also serve as a safety mechanism when the β_1-adrenergic stimulation is desensitized as observed in heart failure [37]. In the same line, purinergic stimulation of a G_i-insensitive adenylyl cyclase could be beneficial for the failing heart featuring an overexpression of G_i [37]. In aging, a process during which the β-adrenergic stimulation is also decreased and whole adenylyl cyclase activity decreased, expression of adenylyl cyclase V increases [38]. Thus, under this physiological state the purinergic stimulation of adenylyl cyclase V could partially rescue the cAMP signal transduction pathway.

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