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Would calcium or potassium channels be responsible for cardiac arrest produced by adenosine and ATP in the right atria of Wistar rats?

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ABSTRACT

Autonomic nerves release ATP, which is processed into adenosine in the synaptic cleft. Adenosine and ATP exert a negative chronotropic effect in the heart. This study aims to evaluate adenosine and P2 receptors and cellular signalling in cardiac arrest produced by purines in the heart. Right atria of adult Wistar rats were used to evaluate the effects of adenosine, ATP and CPA (an adenosine A_1 receptor agonist), in the presence and absence of DPCPX, an adenosine A_1 receptor antagonist. Effects of adenosine A_2 and A_3 receptors agonists and antagonists were also investigated. Finally, involvement of calcium and potassium channels in these responses was assessed using BayK 8644 and 4-Aminopyridine. Cumulative concentration–effect curves of adenosine and CPA resulted in a negative chronotropic effect culminating in cardiac arrest at 1000 μM (adenosine) and 1 μM (CPA). Furthermore, ATP produced a negative chronotropic effect at 1–300 μM and cardiac arrest at 1000 μM in the right atrium. ATPγS (a non-hydrolysable analogue of ATP) reduced chronotropism only. The effects of adenosine, CPA and ATP were inhibited by DPCPX, a selective adenosine A_1 receptor antagonist. The selective adenosine A_2 and A_3 receptors antagonists did not alter the chronotropic response of adenosine. 4-Aminopyridine, a blocker of potassium channels at 10 mM, prevented the cardiac arrest produced by adenosine and ATP, while BayK 8644, activator of calcium channels, did not prevent cardiac arrest. Adenosine A_1 receptor activation by adenosine and ATP produces cardiac arrest in the right atrium of Wistar rats predominantly through activation of potassium channels.

1. Introduction

Isolated right atria have been used as a bona fide model to study cardiac chronotropism given the presence in this tissue of the sinoatrial node (Monfredi et al., 2010), the primary pacemaker of the heart, which is strictly controlled by the sympathetic and parasympathetic nervous systems *in vivo* (Loffelholz and Pappano, 1985).

Terminal endings of the sympathetic and parasympathetic nervous systems release ATP as a co-transmitter of noradrenaline and acetylcholine, respectively. In the synaptic cleft, ATP is converted into adenosine by ectonucleotidases (Vassort, 2001; Zimmermann, 2000).

ATP and adenosine modulate cardiac inotropism and chronotropism (Burnstock and Meghji, 1981; Evans et al., 1982) by activating P2 and adenosine receptors, respectively. Adenosine receptor activation following ATP administration was also observed and attributed to the formation of adenosine after ATP hydrolysis (Moody and Burnstock, 1982). However direct activation of adenosine receptor by ATP in right atria of Wistar rats has not been directly evaluated.

Four subtypes of adenosine receptors have been identified and are ubiquitously expressed in the rat (Dixon et al., 1996). All subtypes are G-protein-coupled receptors (GPCRs) (Fredholm et al., 2001). In cardiac muscle and neurons, adenosine A_1 receptor

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activates pertussis toxin-sensitive potassium channels and K_{ATP} channels and inhibits calcium channels (Jacobson and Gao, 2006). Activation of adenosine A_{2A} receptor leads to increase of adenylyl cyclase activity through preferential activation of Gs protein (Jacobson and Gao, 2006). Adenosine A_{2B} receptors are positively coupled to adenylyl cyclase and phospholipase C (PLC) (Brackett and Daly, 1994). When activated, adenosine A_3 receptor may lead to stimulation of PLC (Abbracchio et al., 1995) or inhibition of adenylyl ciclase (Zhou et al., 1992), both of which result in calcium mobilisation (Shneyvays et al., 2004).

Although adenosine A_1 receptor seems to be the main mediator of the cardiac effects of adenosine (Ford and Broadley, 1997), modulation of cardiac activity by all adenosine receptor subtypes has been shown in different species (Boknik et al., 1997; Salvatore et al., 1993). Studies suggested that adenosine modulates chronotropism in guinea pig right atria through the activation of the adenosine A_3 receptor (Ford and Broadley, 1997). Hove-Madsen et al. (2006) demonstrated that adenosine A_2 receptor activation modulates the frequency of calcium release from sarcoplasmic reticulum in human atrium.

In addition to the potential of adenosine to activate multiple subtypes of receptors in the heart, its downstream signalling molecules remain elusive, in particular the ion channels that mediate adenosine receptor responses. Fassina et al. (1991) reported that adenosine A_1 receptor activation in guinea pig atrium leads to signalling through both calcium and potassium channels. Conversely, Gardner and Broadley (1999) suggested that various agonists of adenosine A_1 receptor may activate both K^+ efflux and calcium channel closure, whereas others may only modulate one channel.

Therefore, this study was carried out in order to: (a) clarify the adenosine receptors involved on the effects of adenosine in the right atrium of Wistar rats; (b) identify the P2 and adenosine receptors involved in the cardiac arrest produced by ATP and adenosine; (c) verify if the chronotropic effects of ATP are due to its conversion into adenosine; (d) identify the ion channels involved in the effects of adenosine and ATP.

2. Materials and methods

2.1. Animal and tissue preparation

Animals were handled and maintained in accordance to protocols approved by the Institutional Research Ethics Committee (protocol 0193/12) at Universidade Federal de São Paulo and following the animal handling procedures conforming to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Council, 2011). Male Wistar rats (*Rattus norvegicus*), albinus variety, 4–6 months-old, from the institutional animal care facility (INFAR-UNIFESP) were killed by decapitation followed by exsanguination under running water. The rib cage was removed to expose the heart and the organ was rapidly removed and placed in Krebs–Henseleit solution (122.3 mM NaCl, 4.6 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4 \cdot 7H_2O$, 17.4 mM $NaHCO_3$; 1.5 mM $CaCl_2H_2O$, 11.1 mM D-glucose, pH 7.4).

The unpaced right atrium was isolated and suspended in a 10 mL organ bath containing Krebs–Henseleit solution, maintained at 36.5 °C and aerated with a mixture of 95% O_2-5% CO_2. Atrium attachment to organ bath and system specifications were similar to that in (Rodrigues et al., 2014).

Tissue was bathed throughout the course of the experiment with Krebs–Henseleit solution and the resting tension for each experiment was standardized using the Frank–Starling's mechanism. The optimal tension obtained was 0.8–1.2 mN, which was used in this study. An equilibration period of 60 min was allowed before starting the experimental protocols (Mustafa et al., 2009).

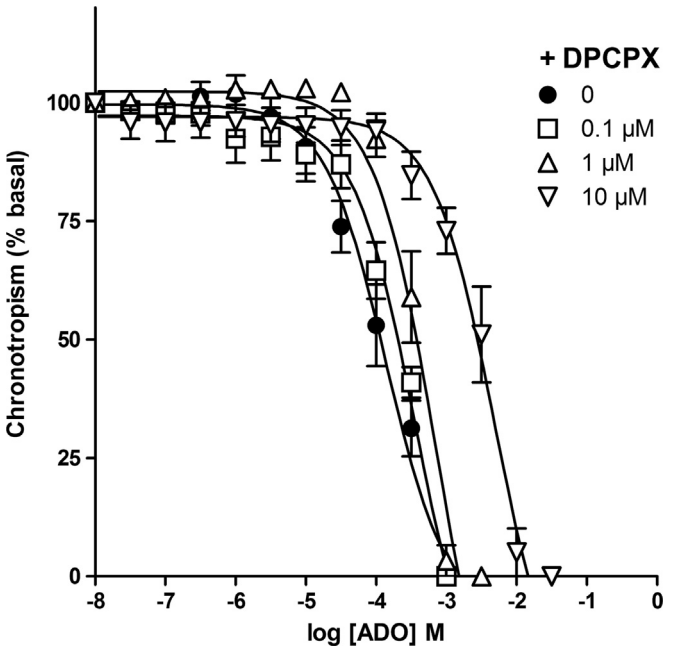

Fig. 1. Role of adenosine A_1 receptors in chronotropic effects of adenosine in rat right atrium. Concentration–effect curve for adenosine (ADO) in right atria in the absence (control curve) or presence of DPCPX (0.1–10 μM), pre-incubated for 20 min. Adenosine produced cardiac arrest. DPCPX caused a right shift in the curve of adenosine in a concentration-dependent manner (one-way ANOVA; $P < 0.05$). Values presented as mean ± S.E.M. of 4–8 experiments.

During the equilibration period tissue was washed every 15 min with fresh KH solution.

2.2. Drugs

The following compounds: adenosine, N^6-Cyclopentyladenosine (CPA), IB-MECA, CGS21680, ATP (adenosine 5′–triphosphate), αβ-MeATP, ATPγS, 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX), 9-Chloro-2-(2-furanyl)-5-((phenylacetyl)amino)-[1,2,4]triazolo[1,5-c]quinazoline (MRS 1220), 4-(-2-[7-amino-2-{2-{furyl}{1,2,4}triazolo2,3-a}{1,3,5} triazin-5-yl amino]ethyl)phenol (ZM 241385), 2–deoxy N^6-methyl adenosine 3,5-diphosphate diammonium salt (MRS2179), 4-Aminopyridine and BayK 8644 were purchased from Sigma-Aldrich (St. Louis, MO, USA). All stock solutions were freshly dissolved in Milli-Q purified water, except for adenosine and DPCPX, which were dissolved in dimethyl sulfoxide. The total volume of dimethyl sulfoxide added never exceeded 0.2% v v^{-1} of the organ bath.

2.3. Experimental protocol

After the equilibration period, agonist concentration–effect curves were performed by cumulative dosing at half-log unit concentration increments. Enough time was allowed for each response to reach the plateau before adding a subsequent agonist concentration. Two concentration–effect curves were constructed in each right atrium.

To determine the pharmacological reactivity to adenosine (0.1 nM to 1000 μM), assays were performed in the absence or presence of three concentrations (0.1, 1 and 10 μM) of DPCPX (a selective adenosine A_1 receptor antagonist), ZM 241385 (a selective adenosine A_2 receptor antagonist) or MRS 1220 (a selective adenosine A_3 receptor antagonist) pre-incubated for 20 min. We also performed cumulative concentration effect curve with the selective adenosine A_1 receptor agonist, CPA (0.1 nM–3 μM), the putative adenosine A_2 receptor agonist, CGS 21680 (0.1 nM to 1000 μM), and the selective adenosine A_3 receptor agonist, IB-

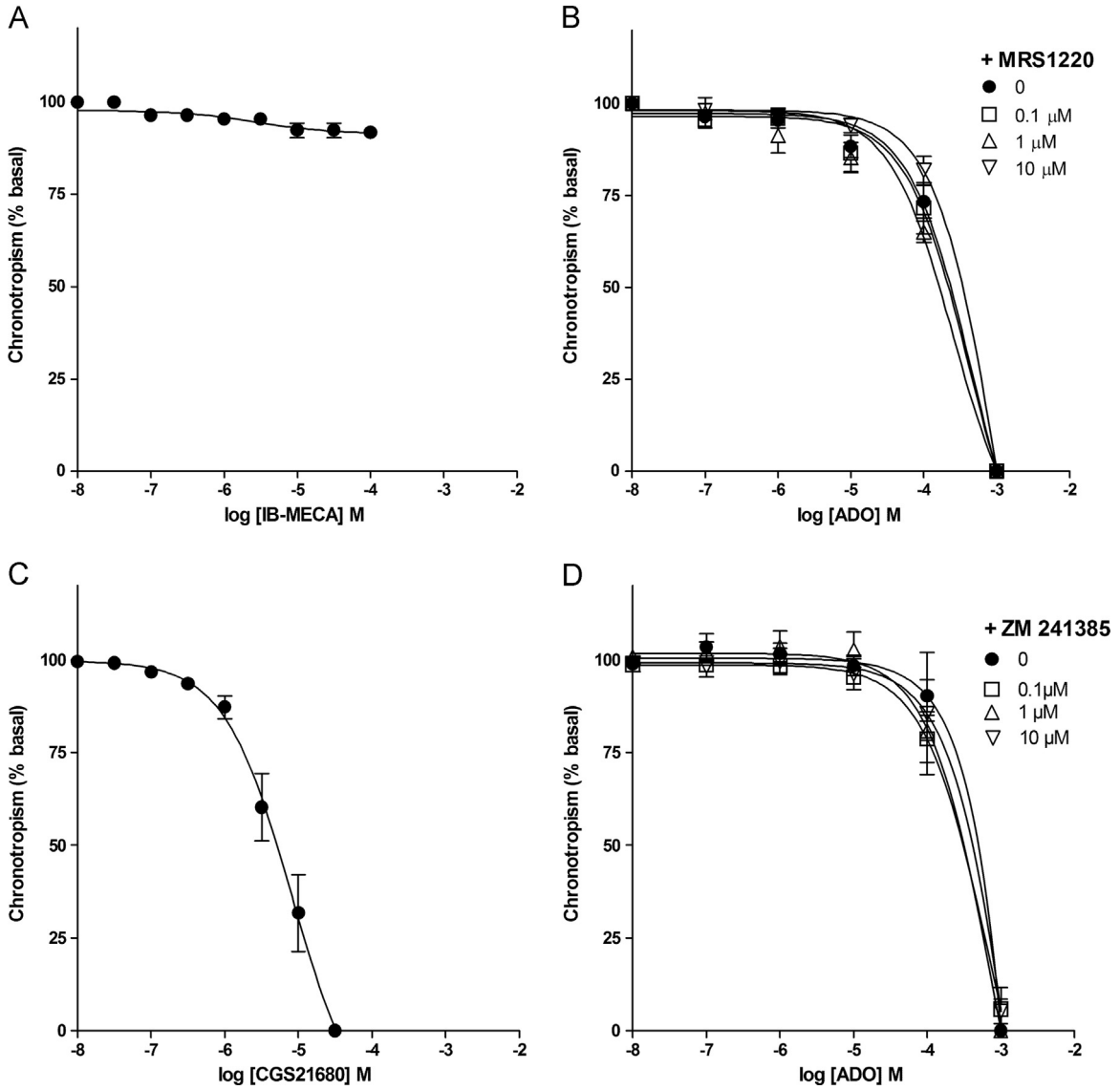

Fig. 2. Role of adenosine A_2 and A_3 receptors in chronotropic effects of adenosine in rat right atrium. (A) Concentration–effect curves for IB-MECA, selective adenosine A_3 receptor agonist. The selective adenosine A_3 receptor agonist did not alter atrial chronotropism (Student's *t*-test, $P > 0.05$). (C) Concentration–effect curves for CGS21680, putative agonist of adenosine A_2 and A_1 receptors. CGS21680 produced a negative chronotropic effect at 0.3 μM and cardiac arrest at 30 μM. (B and D) Concentration–effect curves for adenosine (ADO) in the absence (control curve) or presence of MRS1220 (0.1–10 μM), a selective antagonist of adenosine A_3 receptors, or ZM 241385 (0.1–10 μM), a selective antagonist of adenosine A_2 receptor, respectively. Pre-incubation of MRS1220 or ZM 241385 did not prevent cardiac arrest produced by adenosine (one-way ANOVA; $P > 0.05$). Values presented as mean ± S.E.M. of 5–10 experiments.

MECA (0.1 nM to 1000 μM). Pharmacological reactivity to CPA was also assessed in the presence of three concentrations (1, 3 and 10 nM) of DPCPX pre-incubated for 60 min.

To determine the pharmacological response of rat right atrium to ATP (1–1000 μM), a non-cumulative concentration–effect curve was constructed. Then, the chronotropic regulation of ATP (100 μM) was tested in the presence of PPADS, Suramin (P2Y receptor antagonists) or MRS2179 (a selective $P2Y_1$ receptor antagonist), pre-incubated for 20 min. The atrium was also pretreated with αβMeATP, a desensitizing P2X agonist, and after washout and an equilibration period of 45 min ATP was incubated. We also used ATPγS (100 μM) to distinguish the direct effects of ATP from its metabolites (e.g. adenosine).

Cardiac arrest of adenosine and ATP ($EC_{100} = 1000$ μM) was studied in the presence of the potassium channel blocker 4-aminopyridine (10 mM) or activator of L-type calcium channels BayK 8644 (1–30 μM), pre-incubated for 5 min.

2.4. Pharmacological parameters

The pharmacological parameter E_{max} (maximal effect) refers to the maximum alteration of chronotropism produced by the full agonists used in this work. The potency of agonists (pD_2), measured as the negative log of EC_{50} (Furchgott, 1966), was calculated to allow comparisons between curves of agonists. The pD_2 values were obtained from GraphPad Instat (GraphPad software, USA).

2.5. Statistical analysis

Individual values are presented as mean ± standard error of the mean (S.E.M.), and at least four experiments (n) were performed in each case. Mean values were compared using the conventional analysis of variance for paired samples (Bonferroni post-test) or were subjected to Student's *t*-test. Statistical analysis was performed using GraphPad Instat (GraphPad software, USA). The results were taken to be significant if $P < 0.05$.

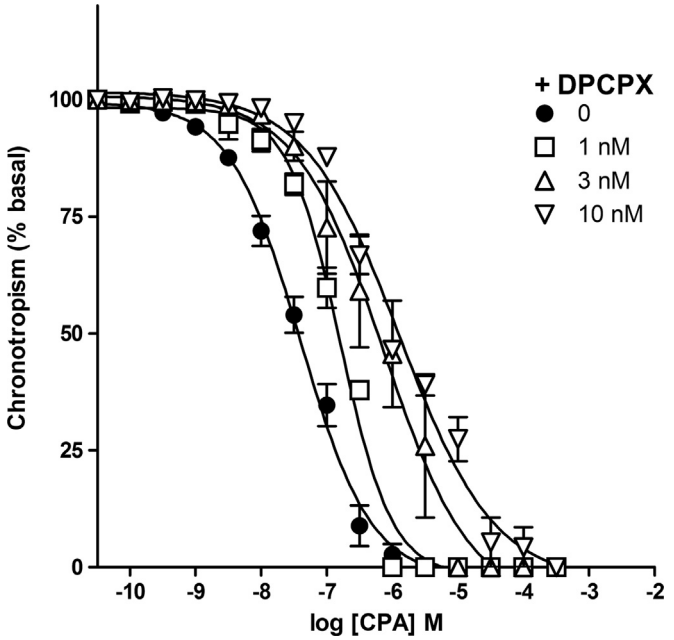

Fig. 3. Selective adenosine A_1 receptor agonist mimic the chronotropic effects of adenosine in rat right atrium. Concentration–effect curves for CPA in right atria in the absence (control curve) or presence of DPCPX (1–10 nM), pre-incubated for 60 min. As observed with adenosine, CPA produced a negative chronotropic effect and cardiac arrest. DPCPX caused a right shift in the curve of CPA in a concentration-dependent manner (one-way ANOVA; $P<0.05$). Values presented as mean ± S.E.M. of 4–8 experiments.

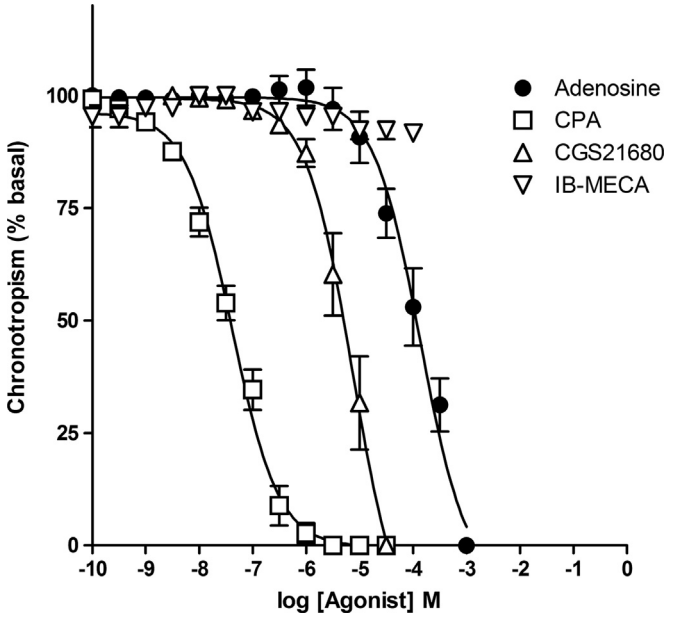

Fig. 4. Adenosine receptor agonists potency study. Chronotropic effect of adenosine receptor agonists in rat right atria. With the exception of IB-MECA, all agonist caused cardiac arrest (one-way ANOVA; $P<0.05$). Values presented as mean ± S.E.M. of 4–10 experiments.

3. Results

3.1. Cardiac arrest in right atria: study of adenosine A_1 receptor

Sinoatrial function was studied using the isolated spontaneously-beating right atrium preparation.

In this model, adenosine promoted a negative chronotropic effect with potency (pD_2) of 4.40 ± 0.16. Moreover, the frequency of spontaneous contractions (274.6 ± 13.8 bpm) was completely abolished (0 bpm) by adenosine at 1000 μM (Fig. 1).

DPCPX, a selective adenosine A_1 receptor antagonist, caused a concentration-dependent inhibition of the effect of adenosine, delaying the onset and reducing the intensity of the negative chronotropic effect at 0.1 μM and 1 μM and preventing cardiac arrest at 10 μM, being necessary to increase adenosine concentration to promote this effect ($P<0.05$; Fig. 1).

Table 1
Pharmacological parameters pD_2 and E_{max} of the adenosine receptor agonists.

Agonist	pD_2	E_{max} (% basal chronotropism)
Adenosine	4.40 ± 0.16	0
CPA	7.56 ± 0.07	0
CGS21680	5.09 ± 0.22	0
IB-MECA	ND[a]	95.73 ± 1.72

[a] As IB-MECA had no effect, pD2 could not be calculated. CPA (N^6-Cyclopentyladenosine, adenosine A_1 receptor agonist); CGS21680 (putative adenosine A_2 receptor agonist); IB-MECA (adenosine A_3 receptor agonist)

3.2. Study of adenosine A_2 and A_3 receptors

To rule out the participation of adenosine A_2 and A_3 receptors, we used agonists of these receptors and evaluated adenosine effects in the presence of their selective antagonists. The selective adenosine A_3 receptor agonist, IB-MECA (0.1 nM to 100 μM), had minimal effect on atrial chronotropism, producing only 4.27 ± 1.72% of inhibition at the highest concentration (100 μM; Fig. 2A).

In addition, MRS 1220 (0.1–10 μM), a selective adenosine A_3 receptor antagonist, did not alter the concentration effect curve of adenosine, and the effect of cardiac arrest was obtained at 1000 μM (Fig. 2B).

Incubation with CGS21680, a putative agonist of the adenosine A_2 receptor (pKi 7.72) and the adenosine A_1 receptor (pKi 5.74), produced a negative chronotropic effect at 0.3 μM and cardiac arrest at 30 μM (Fig. 2C).

Furthermore, ZM 241385 (0.1–10 μM), a selective adenosine A_2 receptor antagonist, did not alter the concentration effect curve of adenosine or the effect cardiac arrest obtained at 1000 μM (Fig. 2D).

3.3. Chronotropic effects of CPA, an adenosine A_1 receptor agonist

To test if the direct activation of the adenosine A_1 receptor negatively regulates heart rate, we performed a cumulative curve with N^6 – Cyclopentyladenosine (CPA), an adenosine A_1 receptor agonist. Similarly to adenosine, CPA produced a negative chronotropic effect with a pD_2 of 7.56 ± 0.07, culminating in cardiac arrest at 1 μM (Fig. 3).

To confirm the selective action of CPA on adenosine A_1 receptors, we pre-incubated DPCPX in a modified protocol, reducing antagonist concentration to 1–10 nM and increasing pre-incubation period to 60 min. DPCPX also inhibited the effects of CPA in a concentration-dependent manner (Fig. 3), however with higher affinity when compared with adenosine assay (Fig. 1).

3.4. Adenosine receptor agonist potency study

The analysis of selective adenosine receptor agonists provides a rank of potency that is characteristic for each receptor subtype. The rank of potency observed was CPA > CGS21680 > ADO»IB-MECA ($P<0.05$, Fig. 4). The E_{max} together with pD_2 for each

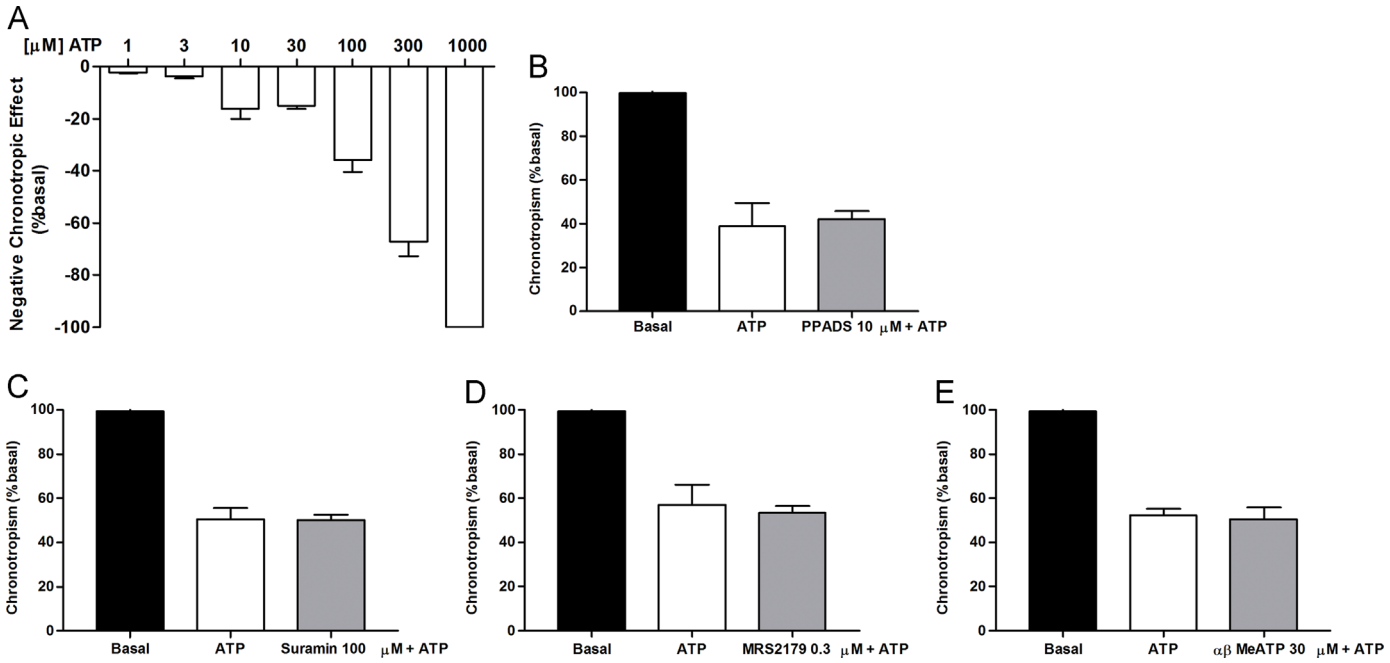

Fig. 5. Role of P_2 receptors in the chronotropic effects of ATP in rat right atrium. (A) Non-cumulative concentration effect curve of ATP (1–1000 μM). ATP produced a concentration-dependent negative chronotropic effect (1–1000 μM) and cardiac arrest at 1000 μM. (B–E) Chronotropic effects of ATP (100 μM) in the presence of PPADS (10 μM), suramin (100 μM), P2Y receptor antagonists, MRS2179 (0.3 μM), $P2Y_1$ receptor antagonist, or after treatment of atrium with the P2X receptor desensitizer agonist αβMeATP (30 μM). Antagonism of P2 receptors did not change the effects of ATP. Values presented as mean ± S.E.M. of 4–7 experiments.

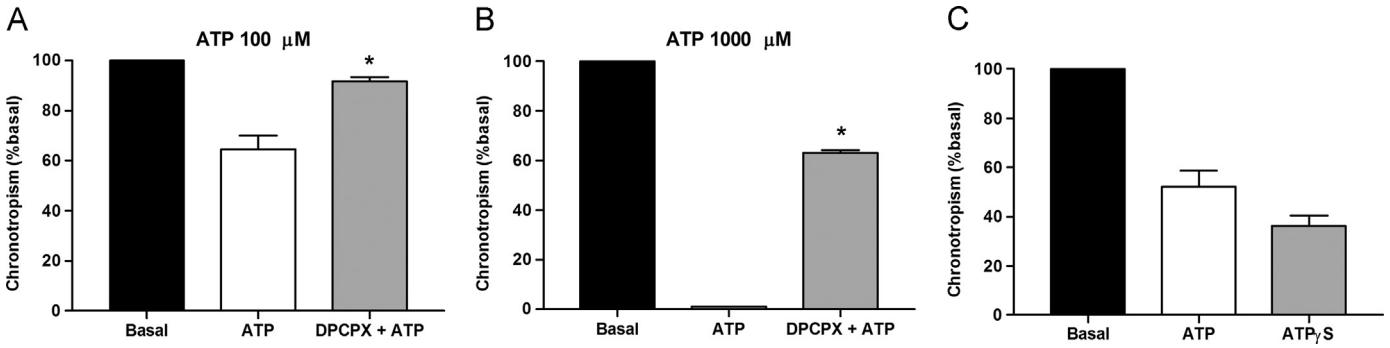

Fig. 6. ATP activates adenosine A_1 receptor directly in rat right atrium. (A) Negative chronotropic effect of ATP (100 μM) is inhibited by DPCPX (1 μM) (Student's *t*-test, $P < 0{,}05$). (B) Cardiac arrest produced by ATP (1000 μM) is also prevented by DPCPX (1 μM) (Student's *t*-test, $P < 0{,}05$). (C) The non-hydrolysable ATP analogue, ATPγS (100 μM), reduced chronotropism equally to ATP (Student's *t*-test, $P > 0{,}05$). Values presented as mean ± S.E.M. of 4–8 experiments.

agonist are reported in Table 1. Together, these data indicate that adenosine A_1 receptor mediates the adenosine chronotropic effects in the rat right atria.

3.5. Study of the effects of ATP in the isolated right atria

In rat right atria, ATP (1–1000 μM) produced a negative chronotropic effect in a concentration-dependent manner. At 1000 μM, ATP also produced cardiac arrest (Fig. 5A).

To identify the receptors involved in the ATP response, we decided to work with the purine at 100 μM (~EC_{50}). Surprisingly, blockade of P2 receptors with PPADS (10 μM), suramin (100 μM) or the selective $P2Y_1$ receptor antagonist MRS2179 (100 nM) did not alter E_{max} of ATP (Fig. 5B–D). Neither did the pre-treatment of atrium with the desensitizer P2X receptor agonist αβMeATP (30 μM) (Fig. 5E).

As these data indicated that P2 receptors are not responsible for the effects of ATP, we assessed if activation of adenosine A_1 receptor was involved in the reduction of chronotropism produced by purines. In the presence of DPCPX (1 μM), the negative chronotropic effect of ATP (100 μM) was practically abolished (Fig. 6A). Furthermore, DPCPX was able to prevent cardiac arrest produced by ATP (1000 μM) (Fig. 6B). Since ATP is rapidly degraded into adenosine, we assessed the chronotropic effects of ATPγS, a non-hydrolysable ATP analogue, on the rat right atrium. ATPγS (100 μM) produced only a negative chronotropic effect, indistinguishable of ATP (Fig. 6C; $P > 0.05$).

3.6. Evaluation of ionic channels participation in adenosine and ATP cardiac arrest

Next, we evaluated the contribution of calcium and potassium channels for cardiac arrest produced by adenosine and ATP. Pre-incubation of right atria with the calcium channel activator BayK 8644 (1 μM) increased atrial chronotropism (Fig. 7A). However, the presence of BayK 8644 (1–30 μM) did not alter the effect of adenosine (1000 μM) or ATP (1000 μM) and cardiac arrest was observed in all the conditions (Fig. 7C).

Pre-incubation with the potassium channel blocker 4-aminopyridine increased atrial chronotropism at 3 mM and E_{max} was obtained at 10 mM (Fig. 7B). The presence of 4-aminopyridine at 10 mM avoided cardiac arrest produced by adenosine and ATP (1000 μM; Fig. 7D).

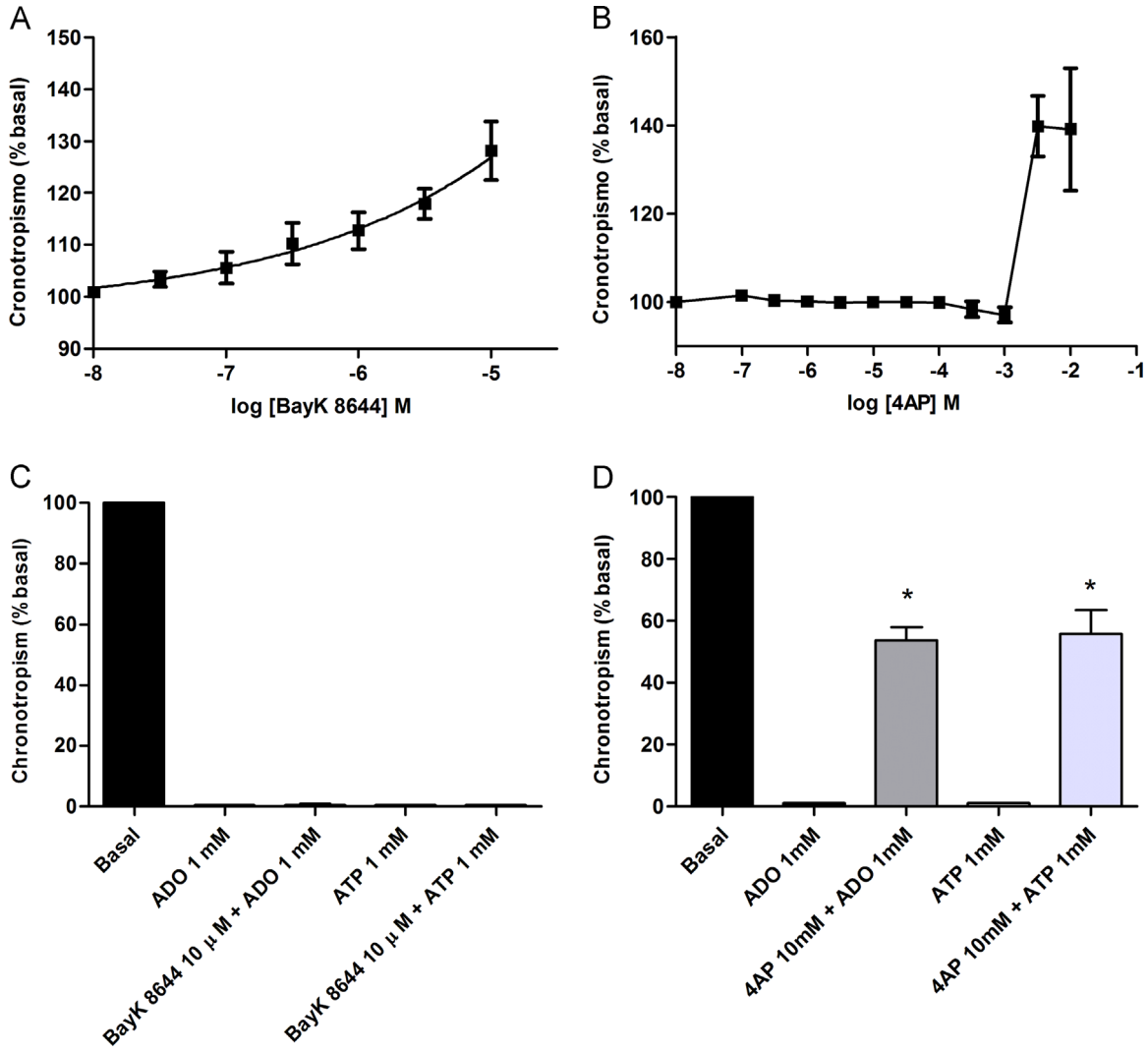

Fig. 7. Evaluation of ion channels involved in cardiac arrest produced by adenosine and ATP. (A and B) Effects of BayK 8644 and 4-aminopiridyne (4AP) on atrial chronotropism. BayK 8644 augmented cardiac chronotropism in a concentration-dependent manner up to the higher concentration used (10 μM). 4-Aminopyridine produced an augmentation of chronotropism at 3 mM, reaching maximum effect at 10 mM. (C and D) Effect of adenosine and ATP (1 mM) on atrial chronotropism of right atria in the absence and presence of BayK 8644 (10 μM) or 4AP (10 mM). BayK 8644 did not prevent cardiac arrest of adenosine or ATP (C). Conversely, 4AP (10 mM) was able to inhibit cardiac arrest produced by adenosine or ATP. Values presented as mean ± S.E.M. of 4–5 experiments (one-way ANOVA; $P < 0.05$).

4. Discussion

Adenosine and ATP modulate chronotropism in right atria as previously described by Rockoff and Dobson (1980) and Pelleg et al. (1990). But the exactly signalling pathways involved in the cardiac arrest remained unknown. Our study provides strong evidence that the cardiac arrest induced by adenosine and ATP is due to activation of potassium channels.

Moreover, the modulation of atrial chronotropism by adenosine (Fig. 1) suggests the presence of functional adenosine receptors in the sinoatrial node of Wistar rats. Reduction of cardiac function by adenosine has been described in frogs (Burnstock and Meghji, 1981), guinea pigs (Meester et al., 1998), humans (Albrecht-Kupper et al., 2012) and rats (Rockoff and Dobson, 1980). Thus, our results are consistent with the modulation of cardiac function exerted by purines.

Some studies suggest that adenosine A_3 receptor is capable of reducing the chronotropism in the heart of rodents and humans (Ford and Broadley, 1997; Headrick and Peart, 2005). In our study, incubation of the right atria of rats with selective adenosine A_3 receptor agonist, IB-MECA, does not alter the chronotropism at the highest concentration (100 μM). In addition, MRS 1220, a selective adenosine A_3 receptor antagonist, does not block the chronotropic effect of adenosine. This suggests that in the right atrium of Wistar rats, adenosine A_3 receptor is not functional.

Hove-Madsen et al. (2006) demonstrated that adenosine A_2 receptor is functional in human atrial myocytes. Therefore we evaluated the involvement of adenosine A_2 receptor in the regulation of atrial chronotropism by employing CGS 21680, a putative agonist of adenosine A_2 (pKi 7.72) and A_1 receptor (pKi 5.74). This drug causes a negative chronotropic effect at 1 μM and cardiac arrest at 30 μM. At this concentration CGS 21680 binds to both adenosine A_2 and A_1 receptor (Liang et al., 2010). As we only noted a decrease of chronotropism, this effect rules out the activation of adenosine A_2 receptor which would lead to an increase of cardiac frequency. Furthermore, the selective adenosine A_2 receptor antagonist (ZM 241385) does not prevent cardiac arrest promoted by adenosine. Therefore we attributed the effect of cardiac arrest produced by CGS 21680 to the selective activation of the adenosine A_1 receptor.

To test the hypothesis that the chronotropic effects of adenosine were mediated by selective activation of adenosine A_1 receptor, we used CPA, an adenosine A_1 receptor agonist. CPA produces negative chronotropic effects on the right atria of Wistar rats, similar to adenosine. The chronotropic effect and cardiac arrest produced by CPA or

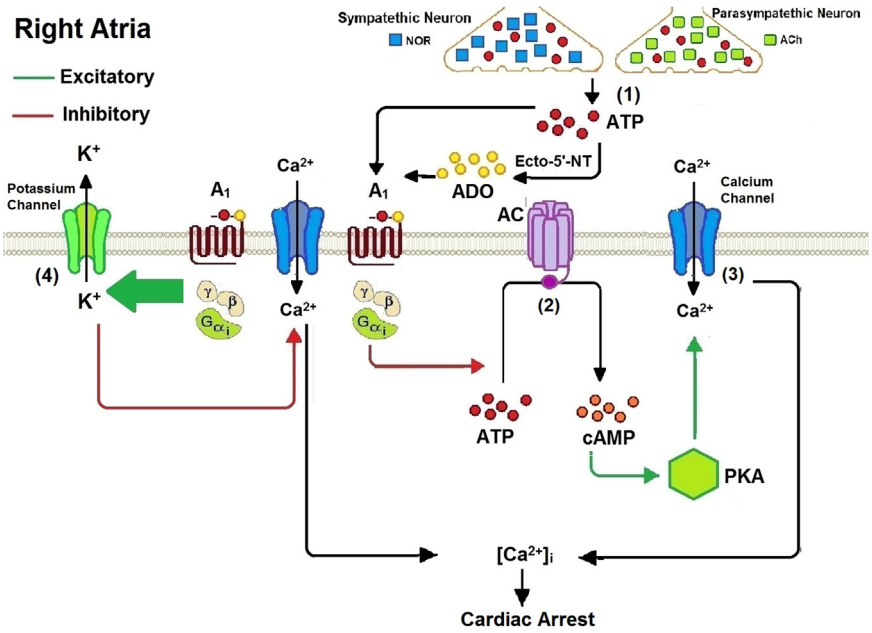

Fig. 8. Schematic model of purinergic system in rat right atria. Adenosine can be obtained by (1) degradation of ATP. Both, adenosine and ATP can activate the adenosine A_1 receptor, which cause cardiac arrest by two pathways: (I) a canonical $G_{i\alpha}$ transduction pathway, with consequent inhibition of AC (2), decrease of cAMP levels and calcium influx (3) and, (II) preferably through opening of potassium channels (4).

adenosine are inhibited by DPCPX, confirming the selective action of these agonists on the adenosine A_1 receptor. Similarly, the activation of the adenosine A_1 receptor by adenosine and stable analogues produced a negative chronotropic effect on guinea pig right atria (Ford and Broadley, 1997).

We observed that ATP reduces the chronotropism at concentrations higher than 1 μM and produces cardiac arrest at 1000 μM in the right atrium of Wistar rats.

To exclude the possibility that these negative chronotropic effects were due to processing of ATP to adenosine we used ATPγS, a non-hydrolysable analogue of ATP. In the right atria, ATPγS induced a negative chronotropic effect only at the maximal concentration (100 μM). This result supports the idea that cardiac arrest produced by ATP is due to direct action of ATP, as observed in the right atria of guinea pigs (Burnstock and Meghji, 1981; Moody et al., 1984).

Moreover, we tested the receptor subtype involved in ATP-induced cardiac arrest. P2 receptor antagonists PPADS and Suramin and even the selective $P2Y_1$ receptor antagonist (MRS2179), which is the P2Y receptor subtype coupled to G_i, (Filippov et al., 2004; Marcet et al., 2004) are unable to prevent the cardiac arrest induced by ATP. Also, the cardiac arrest produced by ATP is still observed after desensitization of P2X receptors by αβMeATP. Only in the presence of DPCPX the cardiac arrest produced by ATP is prevented, confirming the selective role of adenosine A_1 receptor in this phenomenon.

Finally, to clarify the cell signalling pathways involved in the cardiac arrest phenomenon, we used BayK 8644 and 4-aminopyridine, an activator of calcium channels that increases chronotropism in the right atrium (Borea et al., 1989) and a potassium channel blocker, respectively. While pre-incubation of right atrium with BayK 8644 does not prevent cardiac arrest caused by adenosine or ATP, incubation of 4-aminopyridine completely blocks the effect of the purines. This result indicates that adenosine and ATP promote cardiac arrest via the opening of potassium channels. The direct activation of potassium channels through the βγ subunit after the activation of G*i* proteins appears to be the predominant mechanism of signal transduction in the atrial tissue of rats as observed in some studies (Mark and Herlitze, 2000). Furthermore, modulation of cardiac activity by increasing K^+ conductance has been observed in the left atrium (Gardner and Broadley, 1999) and in cultured atrial myocytes of guinea pigs (Belardinelli and Isenberg, 1983), consistent with our findings.

Purines and their analogues are widely used to treat cardiac dysfunctions. For example, adenosine has been clinically used as an anti-arrhythmic drug (Wilbur and Marchlinski, 1997) and, more recently, the use of adenosine A_1 receptor partial agonists has been proposed to improve cardiovascular therapy (Albrecht-Kupper et al., 2012). Therefore, the present study may have clinical impact because it describes new mechanisms of heart rate control by signal transduction through the adenosine A_1 receptor in the right atria of a mammalian model. We also suggest that ATP may directly activate the adenosine receptors, despite the higher affinity for P2 receptors.

5. Conclusion

The findings of the present study support the conclusion that adenosine and ATP mediate negative chronotropic effects and cardiac arrest in the sinoatrial node of Wistar rats due to a selective activation of the adenosine A_1 receptor, which predominantly leads to the opening of K^+ channels (Fig.8).

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Conflict of interest

The authors declare no conflict of interest.

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