Abnormal calcium handling in atrial fibrillation is linked to up-regulation of adenosine A2A receptors

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Aims
Atrial fibrillation (AF) is associated with abnormal sarcoplasmic reticulum (SR) calcium release, which is promoted by adenosine A2A receptor (A2AR) activation. Here, we tested the hypothesis that abnormal calcium release in AF is linked to A2AR remodelling.

Methods and results
Western blotting and quantitative real-time PCR were used to determine A2AR mRNA and protein levels in right atrial samples from patients with and without AF. Effects of A2AR activation on calcium handling were assessed with patch-clamp technique and confocal calcium imaging. A2AR mRNA levels and functional A2ARs were moderately up-regulated in patients with atrial dilation and markedly up-regulated in those with AF. Accordingly, A2AR stimulation significantly increased ryanodine receptor phosphorylation in AF patients, and spontaneous calcium waves increased moderately in myocytes from patients with atrial dilation and strongly in patients with AF (2.2 ± 2.1 to 14.3 ± 8.8 min⁻¹, n = 6, P = 0.01). Moreover, the high baseline level of calcium waves in AF was reduced by A2AR antagonists (3.5 ± 2.0 to 1.3 ± 1.3 min⁻¹, n = 6, P = 0.007) or adenosine deaminase (1.7 ± 1.5 to 0.5 ± 0.6 min⁻¹, n = 10, P = 0.02) suggesting that A2ARs are activated by endogenous adenosine. Indeed, intracellular perfusion with adenosine significantly increased the calcium wave frequency (1.1 ± 0.8 to 8.2 ± 3.3 min⁻¹, n = 8), whereas adenosine removal from the cytosol decreased it (2.1 ± 0.9 to 0.3 ± 0.3 min⁻¹, n = 8, P = 0.04).

Conclusions
Atrial fibrillation patients show increased A2AR expression that may account for the high baseline level of spontaneous SR calcium release seen in myocytes from these patients, and the ability of A2AR antagonists to reduce this abnormal calcium release points to the A2AR as a novel molecular target in AF.

Keywords
Adenosine • Arrhythmia • Atrial myocyte • Ca²⁺ handling • Ryanodine receptor

Introduction
Experimental studies in human atrial tissue have disclosed an association between atrial fibrillation (AF) and abnormalities in intracellular calcium handling. Indeed, human atrial myocytes (HAMs) from patients with AF exhibit a reduced L-type calcium current (I_{CaL}) density¹ and increased spontaneous sarcoplasmic reticulum (SR) calcium release. The latter activates Ca²⁺ extrusion from the cell by the Na-Ca exchanger (NCX), generating an inward NCX current (I_{NCX}) and a concurrent membrane depolarization. Such abnormal depolarizations (afterdepolarizations) are known to favour the occurrence of arrhythmias by triggered

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activity and to promote reinitiation of AF in isolated atrial preparations. The mechanism underlying abnormal SR calcium release in AF is not well known but appears to be linked to an increased cytosolic calcium concentration. Since protein kinase A can mediate RyR2 phosphorylation, it is conceivable that membrane receptors coupled to adenyl cyclase through G protein-coupled receptors modulate intracellular calcium handling. Indeed, A2AR receptors are expressed in human heart atrium and modulate the frequency of spontaneous calcium release. Thus, adenosine-mediated signalling pathways could, in spite of adenosine’s ability to block supraventricular arrhythmias by slowing the AV-nodal conduction, contribute to the initiation of AF and this might explain why administration of an adenosine bolus can induce transient atrial arrhythmias by slowing the AV-nodal conduction. Also, abnormal adenosine levels have been reported during ischaemia and associated to the initiation of AF. In spite of this, investigation has so far focused on remodelling of other cell membrane receptors in patients with AF and it remains to be determined whether AF is associated to remodelling of A2ARs and if such remodelling has any functional consequences.

This study aimed to test the hypothesis that the abnormal SR calcium release seen in patients with AF is linked to remodelling of atrial A2ARs.

Methods

Human samples

Human atrial myocytes were isolated by enzymatic digestion of human right atrial tissue samples as previously described. A total of 254 myocytes from 82 patients undergoing cardiac surgery were used for electrophysiological experiments. To optimize sample quality, samples were obtained from each patient. The study was conducted in accordance with the Declaration of Helsinki principles, and approved by the Ethical Committee of our institution.

Gel electrophoresis, immunoblotting, and quantitative real-time PCR

Membranes from right atrial tissue or HEK cells were prepared as previously described and treated with SDS–PAGE (polyacrylamide gel electrophoresis) sample buffer and resolved by SDS–PAGE using anti-A2AR (1:2000 rabbit polyclonal PA1-042, Affinity BioReagents). To investigate if A2AR stimulation affected RyR2 phosphorylation at ser-2808, each atrial tissue sample was divided in three pieces and for the human glyceraldehyde-3-phosphate dehydrogenase (GAPDH), FGAPDH (5′-GGGGGCTCTCCAGAACATCAT-3′) and RGAPDH (5′-GGGGTCCAAGGGTGCTTACTCC-3′) and for

Table I Clinical data

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No AF, patients without a documented history of AF; AD, patients with atrial dilation; AF, patients with a documented history of AF; Twenty patients had permanent AF and eleven patients had paroxysmal AF; LVEF, left ventricular ejection fraction; CABG, coronary artery bypass graft; ACE-inhibitor, angiotensin converting enzyme inhibitor. Values are given as mean ± standard deviation, number of patients, or percentage (%). A significant difference between the three patient groups (P < 0.05) is indicated with an asterisk.
the human A2AR (hA2A), FhA2A (5′ GGCTGCCCTACACATCAT CAACT 3′) and RhA2A (5′ TGGGCCAGGGGTCACT 3′).

Measurements of spontaneous calcium release and ionic currents in isolated myocytes

Calcium sparks and calcium waves were detected in fluo-4 loaded HAMs at room temperature, using the line scanning mode (Figure 2) or the frame scanning mode (Figure 7) of a Leica TCS SPS AOBS laser scanning confocal microscope. Fluo-4 was excited at 488 nm and fluorescence emission was detected between 500 and 650 nm at scan rates of 1 kHz for line scanning or 100 Hz for frame scanning (each frame was 512 × 140 pixels). Spark detection and ionic current recordings (using a HEKA EPC-10 amplifier) were done as previously described. Briefly, spontaneous $I_{\text{NCX}}$, activated by spontaneous calcium waves, were recorded at −80 mV. The caffeine releasable calcium load was obtained by integration of the $I_{\text{NCX}}$ elicited by rapid exposure to 10 mM caffeine at rest. The ability of the SR to reload calcium was also assessed with a protocol consisting in two caffeine pulses applied immediately before and after a train of 30 stimulation pulses. The first caffeine pulse served to clear the SR calcium content and the second pulse to measure re-loading after the 30 stimulation pulses (used for Figure 3B). The current–voltage relationship for $I_{\text{Ca}}$ was obtained using test potentials between −40 and +50 mV. Steady-state effects on $I_{\text{Ca}}$ were evaluated using repetitive depolarization to 0 mV for 200 ms at 0.5 Hz. The amphotericin perforated-patch configuration was used in all experiments except for Figure 7 where the ruptured patch configuration was used. Recording solutions and criteria for acceptable access resistance have previously been described. No series resistance compensation was employed.

Data analysis and statistics

Experiments were carried out without knowledge about the clinical data of the patients. Unless otherwise stated, average values from

**Figure 1** $A_{2A}$R expression is increased in atrial fibrillation. (A) Immunoblotting with a rabbit anti-$A_{2A}$R antibody against cell membranes from HEK cells transiently transfected with human $A_{2A}$R (C+), human atrial membranes from patients without atrial dilation and AF (no AF, $n = 14$), without AF but with atrial dilation (no AF, AD, $n = 18$), and from atrial fibrillation patients (AF, $n = 8$). (B) Relative Densitometric Scans of immunoblots showing the relative amounts of $A_{2A}$R monomer and dimer normalized to the sum of monomeric and dimeric $A_{2A}$R in no AF patients. Asterisks indicate protein levels that are significantly higher than the corresponding level in samples from patients without atrial fibrillation and without atrial dilation. Hashs indicate a protein level that is significantly higher than the corresponding level in patients with atrial dilation but without atrial fibrillation. (C) Relative $A_{2A}$R mRNA expression normalized to the endogenous control $\alpha$-actinin 1 in 5 no AF patients, in 11 AD patients, and in 8 AF patients. Asterisks indicate mRNA levels significantly higher than the level in patients without atrial dilation and without atrial fibrillation.
each patient were used for statistical analysis and expressed as mean ± standard deviation. Data sets were tested for normality. A two-sided Student’s t-test was used to assess significant differences when testing a specific effect. ANOVA was used for comparison of multiple effects and Student–Newman–Keuls post-test was used to evaluate the significance of specific effects. A χ² test was used to evaluate diagnosis and pharmacological treatments in Table 1. The statistical analysis was done with SPSS Statistics 17.0 and Sigma Stat 3.1 packages. Differences were considered statistically significant for P < 0.05.

Results

Increased A2AR expression in patients with atrial fibrillation

Figure 1A and B shows that both monomeric and dimeric A2AR species are present in human right atrium. The dimeric A2AR species, which is the functional form located on the cell surface,17 was sparse in samples from patients without AF and no AD, while dimeric A2AR expression was moderately higher in patients without AF but with AD, and markedly higher in patients with AF (Figure 1B). Figure 1C shows that also A2AR mRNA, measured by quantitative real-time PCR, increased in patients with AD and with AF.

Effects of A2AR activation on spontaneous sarcoplasmic reticulum calcium release

To assess the potential functional effects of A2AR remodelling, we first analysed the effect of A2AR stimulation on spontaneous SR calcium release (calcium sparks and calcium waves) in isolated HAMs from six patients with and eight patients without AF. Pre-incubation with the A2AR agonist CGS21680, markedly increased both the calcium spark and calcium wave frequency in patients with AF (Figure 2).

Since the increase in spontaneous SR calcium release may result from a direct effect on the RyR2 or from an effect on the SR calcium content18 we also analysed the effect of CGS21680 on the amplitude and decay of 142 calcium sparks from six patients with AF. Neither the amplitude (1.62 ± 0.12 vs 1.59 ± 0.10, P = 0.74) nor the time constant for the decay of the calcium sparks (45 ± 7 vs 52 ± 15 ms, P = 0.14) was altered by CGS21680.

To determine whether the increased spontaneous SR calcium release was secondary to an action on the membrane potential, perforated patch-clamp technique was used to clamp the membrane potential. This did not impede that CGS21680 increased the number of I_{NCX} activated by spontaneous SR calcium release in patients with AF (Figure 3A). Moreover, CGS21680 induced a modest but significant increase in the spontaneous I_{NCX} frequency in myocytes from patients with AD but no AF. Theoretically, A2AR

![Figure 2](image-url)
stimulation may also induce PKA-mediated stimulation of SR calcium uptake and $I_{Ca}$. However, CGS21680 did not modify SR calcium loading at rest (6.9 ± 1.5 vs. 7.0 ± 1.0 amol pF$^{-1}$ with and without CGS21680, $P = 0.83$ in six AF patients) or the re-loading achieved after 30 stimulation pulses (Figure 3B), nor did it change the $I_{Ca}$-voltage relationship (Figure 3C) in patients with or without AF.

**A2AR activation increases RyR2 phosphorylation in fibrillating atria**

To test whether A2AR-activation affects the phosphorylation state of the RyR2, we used a phospho-specific antibody recognizing the RyR2 when phosphorylated at the residue ser-2808. Figure 4 shows that the ratio of ser-2808 phosphorylated RyR2:total RyR2 was significantly higher in atrial tissue samples from patients with AF than from patients without AF. Moreover, A2AR stimulation with CGS21680 increased RyR2 phosphorylation at ser-2808 in AF patients but not in patients without AF. The effect of isoproterenol served as a positive control for phosphorylation of RyR2 at ser-2808. Interestingly, the spontaneous $I_{NCX}$ frequency (Figure 3A) was proportional to the ratio S2808:RyR2 total (Figure 4), being lowest in samples from patients without AF (no AF) incubated with vehicle and highest in samples from patients with AF incubated with CGS21680.

**Effects on sarcoplasmic reticulum calcium handling of A2AR blockade**

To test if the higher incidence of spontaneous calcium release observed in HAMs from AF patients is linked to increased A2AR expression, the effects of A2AR antagonists on SR calcium handling were compared in patients with and without AF. Figure 5A and B shows that A2AR antagonism with ZM241385 reversibly reduced the spontaneous $I_{NCX}$ frequency in AF myocytes to levels observed in myocytes without AF. In contrast, ZM241385 did

![Figure 3](image-url)
not affect $I_{\text{Ca}}$ (Figure 5C and D) or the caffeine-induced $I_{\text{NCX}}$ (97 ± 36% of control in AF, $P = 0.77$ and 101 ± 15% of control in no AF, $P = 0.98$).

**Endogenous adenosine modulate sarcoplasmic reticulum calcium handling through $A_{2A}$R activation**

The inhibition of spontaneous SR calcium release by $A_{2A}$R antagonists suggests that endogenously produced adenosine activate the $A_{2A}$Rs. In agreement with this, exogenous adenosine deaminase (ADA), which degrades the nucleoside, significantly reduced the spontaneous $I_{\text{NCX}}$ frequency, and the non-degradable $A_{2A}$R agonist CGS21680 reversed this effect (Figure 6). Adenosine deaminase had no significant effect on caffeine-induced $I_{\text{NCX}}$ (93 ± 18% of control in AF, $P = 0.34$ and 109 ± 24% of control in no AF, $P = 0.41$).

To verify that endogenous adenosine can stimulate spontaneous calcium release, a subset of myocytes were infused with adenosine through the patch pipette. To track adenosine, 50 μM fluo-4 was included in the pipette solution and the concurrent rise in fluo-4 fluorescence was recorded using confocal calcium imaging (Figure 7A). The time-dependent increase in base-line fluorescence is shown in Figure 7B. Fitting of eight experiments with adenosine infusion gave a plateau of 0.85 ± 0.40 and a time to half-maximum of 15 ± 5 min. The increase in the cytosolic adenosine level can be calibrated (right-hand scale in Figure 7B) if it is assumed to be proportional to the increase in the baseline fluorescence.

As shown in Figure 7C, adenosine infusion initially induced numerous calcium sparks and mini-waves that were gradually replaced by large calcium waves, and the effect of adenosine infusion on the wave frequency is summarized in Figure 7D. Using the calibrated adenosine signal, and fitting this data with a hyperbolic equation (Figure 7D inset), the endogenous adenosine level is estimated to be 0.54 μM (x-offset), and the adenosine concentration for a half-maximal effect is estimated at 4.2 μM. Treatments with adenosine-containing ($n = 8$) and adenosine-free solutions ($n = 8$) had opposite and significantly different effects ($P < 0.05$, one-way ANOVA) on the frequency of calcium waves. In separate experiments, 150 nM extracellular ZM241385 inhibited the effect of internal perfusion with 30 μM adenosine (Figure 7E), confirming that adenosine is activating the $A_{2A}$R.

**Figure 4** Effects of $A_{2A}$R activation on RyR2 phosphorylation at ser-2808. Western blot analysis performed with antibodies for total RyR2 (RyR total) and RyR2 phosphorylation at ser-2808 (S2808) in atrial tissue from patients without (no atrial fibrillation) and with atrial fibrillation. Samples were preincubated in oxygenated Tyrode solution for 20 min in control conditions (CON), with 200 nM CGS21680 (CGS), or with 1 μM isoproterenol (ISO). Densitometric analysis of RyR2 phosphorylation at S2808 relative to total RyR2 is shown. Values for all treatments were significantly higher in atrial fibrillation than the corresponding value in no atrial fibrillation ($P < 0.05$, $n = 9$). A significant difference ($P < 0.05$) from the corresponding control value is indicated with an asterisk above bars.

**Figure 5** Effects of $A_{2A}$R inhibition on spontaneous $I_{\text{NCX}}$. (A) Spontaneous $I_{\text{NCX}}$ before (control), during (ZM241385) and after (wash) exposure of a myocyte to 50 nM ZM241385. (B) $A_{2A}$R inhibition with ZM241385 significantly reduced the $I_{\text{NCX}}$ frequency in myocytes from six patients with atrial fibrillation but not in myocytes from five patients without atrial fibrillation (no atrial fibrillation). The holding potential was −80 mV. Horizontal and vertical scale bars are 30 s and 150 pA. Consecutive traces are separated by 50 pA for clarity. (C) $I_{\text{Ca}}$ recordings from the same patients before (control), during (ZM241385) and after (wash) exposure of a myocyte to ZM241385. Horizontal and vertical scale bars are 200 ms and 125 pA. (D) Average current densities before and after exposure to ZM241385. Where applicable, significant differences are indicated with $P$-values above graph bars.
Discussion

Remodelling of atrial adenosine A2A receptors in atrial fibrillation

In this study, we report a 1.7-fold higher level of A2A mRNA in patients with AD and a four-fold increase in A2A mRNA levels in AF patients, which coincides with corresponding increases in the expression of both the monomeric and the homodimeric form of the A2A. The latter form is predominantly at the cell surface, mediating the signal transduction. Moreover, the overlapping distribution of A2A and RyR2s in HAMs likely facilitates A2A-mediated stimulation of spontaneous SR calcium release. These findings suggest that AD and particularly AF have the potential to induce remodelling of A2A Rs. The mechanism underlying the observed upregulation of A2A Rs is unknown, but AF has been associated with energetic deficits, which could modulate adenosine receptor expression. Moreover, the structural protein α-actinin located at the z-lines is known to interact with the A2A and modify its expression at the cell membrane, and the AD present in the AD patients and AF patients could impose a mechanical stress on α-actinin and in turn modify A2A expression. In line with the observed remodelling of A2A Rs in AF, changes in the expression of other membrane receptors have also been reported in patients with AF; including angiotensin receptors, opioid receptors, and 5-HT4 receptors. The concurrent remodelling of the different receptors may give rise to variable or even opposite effects on intracellular calcium handling. Indeed, upregulation of angiotensin, 5-HT4, and IP3 receptors in AF is expected to promote intracellular calcium mobilization, whereas down-regulation of 5-HT4 receptors is expected to reduce lca, amplitude and SR calcium loading.

Remodelling of adenosine A2A receptors linked to abnormal calcium handling

Abnormal SR calcium release, a condition that favours the occurrence of arrhythmogenic afterdepolarizations, has previously been recognized in isolated right atrial myocytes from patients with AF and attributed to an increased RyR2 activity. The specific mechanism underlying the association between AF and abnormal cellular calcium handling has not yet been elucidated, but the present data provide evidence for a functional link to atrial A2A remodelling. Thus, the natural ligand, adenosine, and selective A2A agonism increased spontaneous calcium release moderately in patients with AD and strongly in AF patients while selective A2A antagonism had the opposite effect in AF patients. There are several ways by which A2A stimulation could promote spontaneous calcium release from the SR: (i) by directly affecting the RyR2 activity, (ii) by increasing the SR calcium content, or (iii) by promoting cross-talk between IP3 receptors and RyRs. Our data indicate that the most likely mechanism for the increased SR calcium release in AF is an increased RyR2 phosphorylation. Thus, the ratio of phosphorylated RyR2 at ser-2808:total RyR2 was higher in AF patients than in patients without this arrhythmia, and A2A stimulation significantly increased RyR2 phosphorylation at ser-2808 in patients with AF.

In contrast, our findings do not support a substantial A2A-mediated modulation of the lca, amplitude, the amplitude or decay of calcium sparks, the amplitude of spontaneous ln, or the SR calcium content. These findings indicate that A2A activation does not modify calcium extrusion on each spontaneous ln, and A2A activation is therefore unlikely to increase calcium entry between two successive ln. Furthermore, CGS21680 had a comparable effect in clamped (Figure 3A) and unclamped cells (Figure 2C), ensuring that the effect was not secondary to an effect on the resting membrane potential, which is known to modulate spontaneous SR calcium release. In agreement with these findings, A2A stimulation increased cyclic AMP content but not phospholamban phosphorylation in guinea pig ventricle.

Limitations of the model

A concern when interpreting data from human cardiomyocyte models is that clinical or therapeutic heterogeneity among patients with and without AF can potentially bias the results. To minimize this, patients treated with calcium antagonists were excluded from this study. Also, there is little evidence in the literature that the treatments that differed among patients with and without AF (statins, diuretics, acetyl salicylic acid, or dicoumarin, see Table 1) alter calcium handling in isolated cardiomyocytes. Moreover, unpublished observations from our laboratory show that these treatments do not affect lca, the caffeine releasable calcium load, or the spontaneous ln frequency in HAMs. Moreover, the observed remodelling of intracellular calcium handling is consistent with previous reports on human AF. The stimulatory
The arrhythmogenic potential of spontaneous calcium release in HAMs is based on reports showing involvement of the SR in the induction of afterdepolarizations and reinitiation of AF. Likewise, spontaneous SR calcium release favours abnormal automaticity, another mechanism contributing to atrial arrhythmias in humans. Thus, abnormal SR calcium release may contribute to the initiation or recurrence of AF, and our study suggests that increased A2AR expression in AF patients is a novel mechanism that may promote domestication of the arrhythmia by increasing spontaneous calcium release at baseline. Moreover, local atrial ischaemia can contribute to the development of AF and a dramatic increase in interstitial adenosine levels has been observed during myocardial ischaemia. In fact, our estimates of the (adenosine) at baseline (0.54 μM) and at half-maximal activation of calcium waves (4.2 μM) are well within the levels reported before and during ischaemia and 4.2 μM is below levels reached when an adenosine bolus is used in clinical practice to induce ectopic atrial activity, suggesting that periods of deficient circulation in atrial appendices of AF patients has the potential to perpetuate AF through A2AR-mediated stimulation of spontaneous SR calcium release. Adenosine is also known to inhibit AV-nodal conduction and shorten the atrial refractory period. The latter favours electrical re-entry and has been attributed to adenosine A1 receptor activation and proposed to account for adenosine’s proarrhythmic effect. Theoretically, activation of adenosine A3 receptors could produce a similar effect, but this receptor is not expressed in murine atria. The A2AR-mediated effects reported here are expected to have little effect on the refractory period, and likely represent a novel mechanism of particular relevance to the induction of ectopic activity and the (re)-initiation of AF. Moreover, the moderate remodelling of A2AR signalling observed in patients without AF but with AD is in line with a moderate electrical remodelling reported in a sheep model of AD and in patients with high risk of AF.

**Clinical implications**

The arrhythmogenic potential of spontaneous calcium release in HAMs is based on reports showing involvement of the SR in the induction of afterdepolarizations and reinitiation of AF. Likewise, spontaneous SR calcium release favours abnormal automaticity, another mechanism contributing to atrial arrhythmias in humans. Thus, abnormal SR calcium release may contribute to the initiation or recurrence of AF, and our study suggests that increased A2AR expression in AF patients is a novel mechanism that may promote domestication of the arrhythmia by increasing spontaneous calcium release at baseline. Moreover, local atrial ischaemia can contribute to the development of AF and a dramatic increase in interstitial adenosine levels has been observed during myocardial ischaemia. In fact, our estimates of the (adenosine) at baseline (0.54 μM) and at half-maximal activation of calcium waves (4.2 μM) are well within the levels reported before and during ischaemia and 4.2 μM is below levels reached when an adenosine bolus is used in clinical practice to induce ectopic atrial activity, suggesting that periods of deficient circulation in atrial appendices of AF patients has the potential to perpetuate AF through A2AR-mediated stimulation of spontaneous SR calcium release. Adenosine is also known to inhibit AV-nodal conduction and shorten the atrial refractory period. The latter favours electrical re-entry and has been attributed to adenosine A1 receptor activation and proposed to account for adenosine’s proarrhythmic effect. Theoretically, activation of adenosine A3 receptors could produce a similar effect, but this receptor is not expressed in murine atria. The A2AR-mediated effects reported here are expected to have little effect on the refractory period, and likely represent a novel mechanism of particular relevance to the induction of ectopic activity and the (re)-initiation of AF. Moreover, the moderate remodelling of A2AR signalling observed in patients without AF but with AD is in line with a moderate electrical remodelling reported in a sheep model of AD and in patients with high risk of AF.
suggesting that A$_{2A}$AR-linked changes in calcium handling precedes AF. Prevention of AF recurrences by A$_{2A}$AR antagonism has not yet been assessed but the ability of A$_{2A}$R antagonist to inhibit spontaneous SR calcium release without compromising $I_{Ca}$ or the SR calcium content affords a pathophysiological rationale for such an approach to be attempted.

In summary, this is to the best of our knowledge, the first study to demonstrate a functional link between high baseline levels of spontaneous calcium release in patients with AF and increased A$_{2A}$R expression, and RyR phosphorylation. These findings propose abnormal A$_{2A}$R activation in AF as a novel mechanism contributing to the domestication of AF. Moreover, the selective reduction in spontaneous calcium release by A$_{2A}$R antagonists observed in myocytes from patients with AF affords a pathophysiological rationale for testing A$_{2A}$R blockade as a new therapeutic approach to prevent recurrence of AF.

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References