

# Adenosine Induces Ventricular Arrhythmias in Hearts With Chronic Chagas Cardiomyopathy

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Adenosine released during ischemia and hypoxia can induce ventricular arrhythmias. This phenomenon is also observed in Chagas disease. This study involved pharmacologic analysis of the arrhythmogenic properties of adenosine in healthy Sprague-Dawley rats (n=14) and in rats with chronic Chagas cardiomyopathy (n=14). Electrocardiographic and pharmacologic studies were performed on isolated hearts prepared using the Langendorff method. Adenosine increased ventricular arrhythmias in both groups of animals in a dose-dependent manner: 50% of chagasic rats developed ventricular fibrillation compared with 7.14% of healthy rats ( $P < .05$ ). Fibrillation was prevented by A1 (i.e., DPCPX) and A2a (i.e., 8-CSC) receptor antagonists. Arrhythmia was associated with a prolonged QT interval, early depolarization, and the R-on-T and torsade de pointes phenomena. In conclusion, adenosine is a proarrhythmic drug that is able to induce ventricular fibrillation in chagasic rat hearts.

**Key words:** Chagas disease. Adenosine. Arrhythmia. Langendorff system.

## Adenosina induce arritmias ventriculares en corazones con miocardiopatía chagásica crónica

La adenosina liberada durante la isquemia y la hipoxia es capaz de inducir arritmias ventriculares, fenómenos también observados en la enfermedad de Chagas. En este trabajo caracterizamos farmacológicamente las propiedades arritmogénicas de la adenosina en ratas Sprague Dawley sanas (n = 14) y con miocarditis chagásica crónica (n = 14), que fueron sometidas a estudios electrocardiográficos y farmacológicos en preparaciones de corazón aislado tipo Langendorff. La adenosina incrementó las arritmias ventriculares en ambos grupos en forma dosis-dependiente, y en un 50% de las ratas chagásicas indujo fibrilación ventricular respecto a un 7,14% de las ratas sanas ( $p < 0,05$ ). La fibrilación fue prevenida por antagonistas A1 (DPCPX) y A2a (8-CSC). Las arritmias se asociaron a un QT prolongado, a depolarizaciones tempranas, a fenómenos R sobre T y *torsade de pointes*. En conclusión, la adenosina es un fármaco proarrítmico capaz de inducir fibrilación ventricular en ratas chagásicas.

**Palabras clave:** Chagas. Adenosina. Arritmias. Sistema Langendorff.

## INTRODUCTION

The heart is the main organ affected by Chagas disease. Some 20%-30% of patients suffer chronic chagasic cardiomyopathy (CCC), characterized by heart failure, arrhythmias and sudden death. Microvascular lesions have been implicated in the

pathogeny of CCC; these cause multiple ischemic foci,<sup>1</sup> leading to an increase in the concentration of adenosine, the cardiovascular effects of which are mediated by G protein-coupled receptors.<sup>2</sup>

The therapeutic properties of adenosine have been associated with proarrhythmic effects. In patients with structurally normal hearts, adenosine can induce atrial fibrillation, an increase in atrioventricular (AV) node conduction, and nonsustained polymorphic ventricular tachycardia.<sup>3</sup> In children and adults with prolonged QT syndrome, it can induce torsade de pointes (TP)-type ventricular tachycardia.<sup>4</sup>

Sudden death in Chagas disease is owed to ventricular fibrillation (VF) conditioned by humoral, vascular and structural anomalies. Ventricular fibrillation has been related to ventricular repolarization abnormalities reflected in a long QT

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interval<sup>5</sup> and early- (the R- on- T phenomenon) and late-coupled premature ventricular complexes.<sup>6</sup> Focal myocardial ischemia in Chagas disease induces the release of adenosine, which unleashes fatal arrhythmias facilitated by repolarization abnormalities. The present work investigates the role of the adenosinergic system in inducing of ventricular arrhythmia.

## METHODS

### Sample

Twenty-eight Sprague-Dawley rats were divided into 2 groups: a control group of healthy rats (n=14) and a group with CCC (n=14; confirmed histopathologically to have chronic myocarditis) (Figure 1).

### Isolated Heart

A Langendorff continuous retrograde perfusion system was set up, involving a modified Krebs-Henseleit-type solution containing 10 nmol of isoproterenol, pH 7.35-7.45, bubbled with 95% O<sub>2</sub> and 5% CO<sub>2</sub> at 37°C. The isoproterenol was used to maintain high adrenergic tone and to facilitate the appearance of arrhythmias. Electrocardiographic readings were taken in bipolar mode; the electrodes were located at the tip of the right atrium. The effect of adenosine was tested at increasing concentrations (10-3333 μmol). The effects of selective A1 (1,3-dipropyl-8-cyclopentylxanthine [DPCPX]), A2a (8-chlorostyryl caffeine [8-CSC]) and A2b (alloxazine [ALLO]) receptor antagonists, and the A3 antagonist

VUF 5574, were assayed in the presence and absence of 0.33-1 mmol adenosine.

### Data Analysis

Data were analyzed using the Student *t* test or ANOVA for paired samples as required, followed by the Bonferroni test. Qualitative variables were analyzed using the  $\chi^2$  test. Significance was set at  $P < .05$ .

## RESULTS

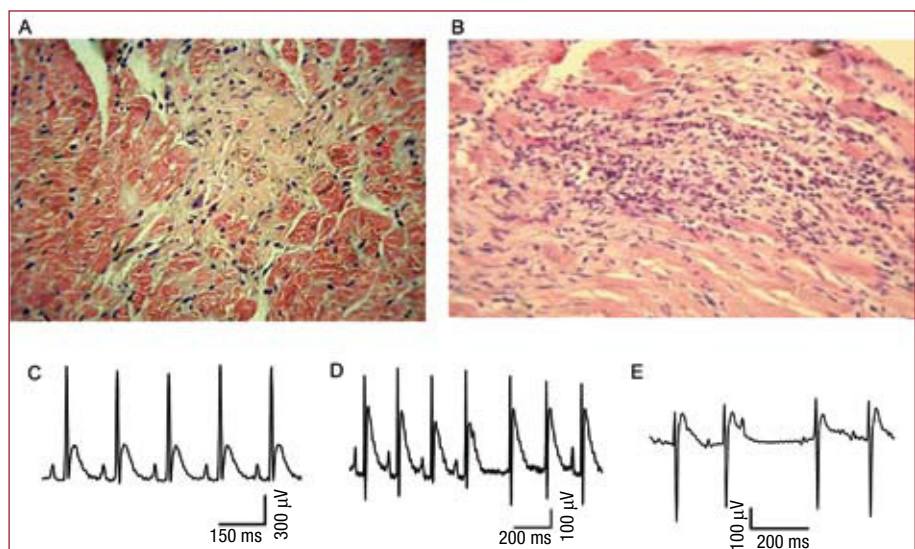
Electrocardiograms were recorded with pentobarbital anesthesia before setting up the Langendorff preparation; these showed chagasic heart rhythm abnormalities (nodal extrasystoles) in five rats, as well as conduction abnormalities (AV and fascicular block). Three control rats showed RR variability only (Figure 1).

### Electrocardiographic Variables

Tables 1 and 2 show the PR, QRS and QT intervals obtained with increasing doses of adenosine and with DPCPX in the presence of 333 μmol of adenosine. The results show that adenosine induced an increase in the QT interval that was reverted with DPCPX. No significant differences were seen for the PR and QRS intervals.

Figure 2A shows the increase in the QT interval and the presence of early depolarizations (arrow). Figure 2B shows premature ventricular complexes emerging from the T wave. Figure 2C shows the TP phenomenon emerging from a prolonged T wave.

**Figure 1.** Histopathological and electrocardiographic evidence of chronic chagasic cardiomyopathy. The sampled heart sections were fixed in PBS-formalin, set in paraffin blocks and stained with hematoxylin and eosin. A shows fibrosis; B shows the mononuclear inflammatory infiltrate. The examples shown are for healthy (C) and chagasic rats (D-E) anesthetized with pentobarbital (40 mg/kg) before the extraction of the heart. Notice that the chagasic rats show nodal extrasystole (D) and atrioventricular block (E).



**TABLE 1. Effect of Adenosine on the Electrocardiographic Variables of Healthy and Chagasic Rat Hearts**

[Ado],(μmol)	PR, ms		QRS, ms		QT, ms	
	Control	Chagasic	Control	Chagasic	Control	Chagasic
0	53.78 (1.82)	57.3 (5.1)	15 (1.41)	16.9 (1.31)	132.1 (4.67)	122.5 (5.26)
10	55.78 (2.42)	61.3 (7.36)	15.89 (1.48)	17.8 (1.42)	150.8 (3.71)	132.3 (10.65)
33	52 (2.45)	57.8 (5.13)	16 (1.43)	17.5 (1.52)	159.6 (5.73)	149.6 (17.74)
100	55.67 (1.99)	60.44 (2.97)	17.11 (1.72)	17.11 (1.52)	167.2 (10.5)	144.5 (21.08)
333	55.44 (3.62)	59.44 (2.18)	15.89 (0.68)	16.78 (1.61)	214.2 (24.49) <sup>a</sup>	161 (23.83) <sup>a</sup>
1000	54.83 (3.15)	60.5 (3.38)	16.13 (1.08)	17.38 (1.49)	234.1 (23.1) <sup>a</sup>	179.4 (23.19) <sup>a</sup>

Ado indicates adenosine. <sup>a</sup>*P*<.05 with respect to Ado 0 μmol.

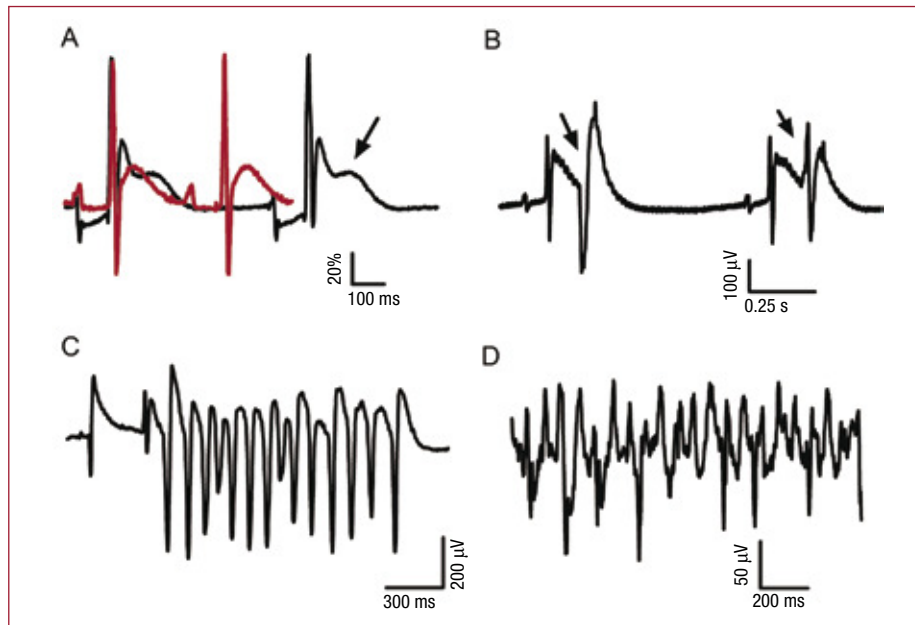
**TABLE 2. Effect of DPCPX in the Presence of Adenosine on the Electrocardiographic Variables of Healthy and Chagasic Rat Hearts**

[DPCPX] (nmol)	PR, ms		QRS, ms		QT, ms	
	Control	Chagasic	Control	Chagasic	Control	Chagasic <sup>a</sup>
0	54.14 (2.86)	56.17 (3.59)	17.33 (1.01)	17 (1.53)	258.3 (30.95)	174.8 (29.18)
100	57.38 (4.58)	58.5 (2.26)	18.44 (0.6)	17.83 (1.45)	220.8 (22.73)	179.3 (29.87)
333	55 (4.19)	57.33 (3.31)	18 (0.85)	17.33 (1.96)	203.1 (20.59) <sup>b</sup>	173.1 (31.24)
1000	59.22 (2.28)	60.14 (2.4)	17.56 (1.59)	18.57 (1.72)	185.8 (19.48) <sup>b</sup>	148.1 (18.22)
3333	61.67 (1.86)	58.71 (1.41)	16.89 (1.53)	17.71 (1.86)	169.7 (23.07) <sup>b</sup>	128.6 (15.64)

DPCPX indicates 1,3-dipropyl-8-cyclopentylxanthine.

<sup>a</sup>*P*<.05 by ANOVA for paired samples; however, the Bonferroni test detected no significant differences between the doses tested.

<sup>b</sup>*P*<.05 with respect to Ado 0 μmol. 0 represents perfusion with 333 μmol adenosine in the absence of DPCPX.



**Figure 2.** Prolongation of the QT interval induced by early post-depolarizations, R on T phenomena, torsade de pointes and ventricular fibrillation. A: recordings obtained for chagasic hearts in the presence (black line) and absence (red line) of 100 μmol adenosine; note the prolongation of the QT interval and early depolarizations (arrow); the amplitude was normalized to facilitate the superimposition of the recordings. B: premature ventricular complexes arising out of the T wave (R on T phenomenon). C: torsade de pointes. D: ventricular fibrillation.

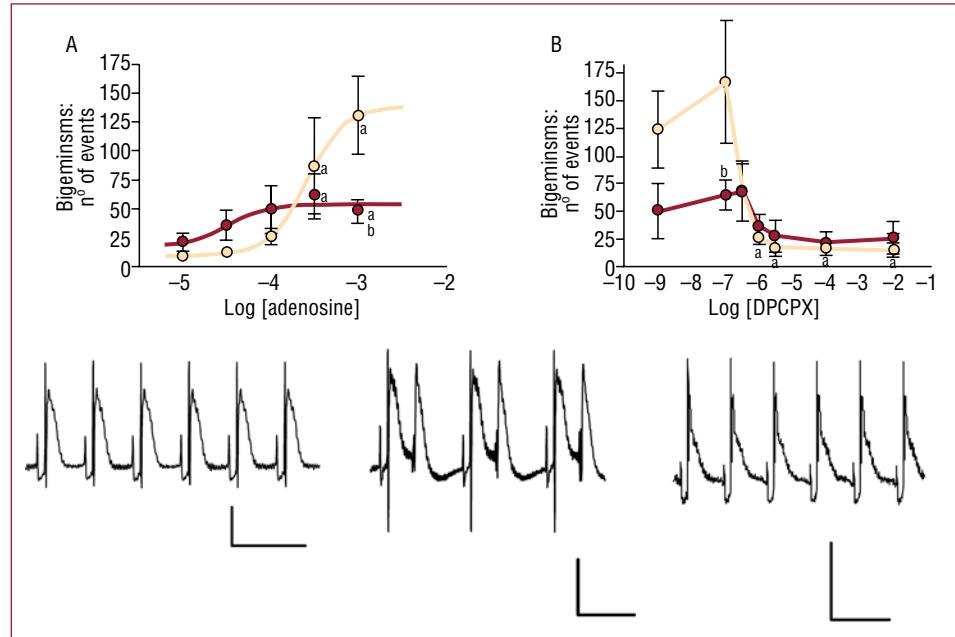
### Rhythm Abnormalities

Adenosine induced a significant increase in the number of nodal and ventricular extrasystoles and bigeminisms in both groups in a dose-dependent manner; these reverted with DPCPX. The members of the control group suffered more bigeminisms (*P*<.05) (Figure 3).

### Ventricular Fibrillation

Adenosine induced VF in 50% (n=7) of chagasic hearts and 7.14% (n=1) of healthy hearts (Figure 2D). The chagasic hearts were associated with the possibility of fibrillation (*P*<.05). No VF was seen in the presence of DPCPX and 8-CSC, but 8-CSC induced ventricular tachycardia in 50% (n=2) of

**Figure 3.** Bigeminsms induced by A1 receptors. Beige represents the control, red the treatment. All recordings were obtained from the same chagasic rat. A: adenosine dose-response curve. B: dose response curve for DPCPX in the presence of 333  $\mu$ mol adenosine; recordings are baseline (left), perfusion with adenosine (middle) and perfusion with DPCPX-adenosine (right); the vertical line represents 100  $\mu$ V and the horizontal 0.5 s. <sup>a</sup> $P < .05$  with respect to baseline. <sup>b</sup> $P < .05$  between groups at the dose indicated.



the hearts. Neither alloxazine 1 mmol nor VUF 176 nmol prevented VF.

## DISCUSSION

Congenital<sup>4</sup> and acquired<sup>7</sup> prolonged QT syndrome is a predisposing factor to serious, adenosine-induced ventricular tachycardia. In such patients the bradycardia induced by adenosine causes TP, which can degenerate into ventricular fibrillation.<sup>8</sup> Certainly, TP is an uncommon type of arrhythmia, and its appearance depends on the existence of bradycardia<sup>9</sup> and increased adrenergic discharge.<sup>10</sup>

The intracellular overload of  $Ca^{2+}$  induced by isoproterenol<sup>11</sup> and adenosine, mediated by the A2a receptors<sup>2</sup> activates the  $Na^+/Ca^{2+}$  exchange mechanism, which in turn prolongs the QT interval. Such conditions would favor the appearance of early and late depolarizations. The former are exacerbated by low heart rates and contribute to the appearance of polymorphic ventricular arrhythmias associated with a long QT interval.<sup>11</sup> It is known that the simultaneous administration of adenosine and isoproterenol can induce arrhythmias in patients prone to atrial fibrillation.<sup>12</sup>

In the present work, the administration of DPCPX led to a significant reduction in the length of the QT interval and the reversion of arrhythmia. The relationship between the A1 receptors and the induction of arrhythmias has been demonstrated in transgenic models.<sup>13</sup>

Adenosine was able to induce VF in the chagasic rats, an effect avoided with the administration of DPCPX and 8-CSC, indicating the mediation

of the A1 and A2a receptors. It is known that the probability of VF appearing, facilitated by hypoxia and reoxygenation phenomena, is reduced with DPCPX,<sup>14</sup> confirming that the A1 receptors are involved in VF. It is also known that the ability of adenosine to induce VF is blocked by the depletion of catecholamines in the heart,<sup>15</sup> confirming that adenosine induces VF under hypoxia-reoxygenation conditions via a mechanism facilitated by the activation of the adrenergic system.

In CCC, abnormalities of the coronary microcirculation lead to cycles of hypoxia, ischemia and reoxygenation.<sup>1</sup> This would condition the chagasic heart to the profibrillatory action of adenosine, which, in the present model, was favored by the sustained activation of the beta-adrenergic receptors. The medical importance of this work lies in its linking of the arrhythmias associated with the prolonged QT interval with the A1 adenosinergic system. This opens up the possibility of studies designed to investigate the therapeutic effect of A1 antagonists in these arrhythmias.

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