

Cardiomyocyte dysfunction during the chronic phase of Chagas disease

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Chagas disease, which is caused by the parasite Trypanosoma cruzi, is an important cause of heart failure. We investigated modifications in the cellular electrophysiological and calcium-handling characteristics of an infected mouse heart during the chronic phase of the disease. The patch-clamp technique was used to record action potentials (APs) and L-type Ca²⁺ and transient outward K⁺ currents. [Ca²⁺]_i changes were determined using confocal microscopy. Infected ventricular cells showed prolonged APs, reduced transient outward K⁺ and L-type Ca²⁺ currents and reduced Ca²⁺ release from the sarcoplasmic reticulum. Thus, the chronic phase of Chagas disease is characterised by cardiomyocyte dysfunction, which could lead to heart failure.

Key words: Chagas disease - calcium current - action potential - potassium current - intracellular calcium - *Trypanosoma cruzi*

Chagas disease, which is caused by infection with the protozoan parasite *Trypanosoma cruzi*, is one of the leading causes of heart failure in Latin America (Marin-Neto et al. 2007). Currently, 10 million people are infected and 25 million people live in areas where the infectious parasites are present (WHO 2010). However, until now, few studies have provided compelling evidence of cardiomyocyte dysfunction during the establishment of heart failure following infection by *T. cruzi* (de Carvalho et al. 1992, Pacioretty et al. 1995, Roman-Campos et al. 2009b). Recently, new data have provided supporting evidence of how the left ventricular electrical-mechanical dysfunction observed during the initial stages of chagasic cardiomyopathy occurs (Esper et al. 2012, Roman-Campos et al. 2012). However, the cellular mechanisms underlying cardiomyocyte dysfunction during the late stages of Chagas disease remain unknown. Therefore, we sought to determine whether cardiomyocyte function is altered in the late phase of Chagas disease.

Eight-week-old male C57BL/6 mice were intraperitoneally infected with 50 bloodstream trypomastigote forms of a Colombian *T. cruzi* strain (Federici et al. 1964). The experimental protocols were approved by the local animal use and care committee of the Federal University of Minas Gerais.

Adult left ventricular myocytes were enzymatically isolated using collagenase (1 mg/mL) with a calcium gradient method as previously described (Roman-Campos et al. 2009b). Myocytes were freshly isolated and

stored in Dulbecco's Modified Eagle's Medium until they were used for experiments (within 4-6 h). Only calcium-tolerant, quiescent, rod-shaped myocytes showing clear cross-striations were used.

The whole-cell patch-clamp method was used in the current and voltage-clamp modes. All experiments were performed at room temperature (23-26°C). To measure action potentials (APs) and transient outward K⁺ (I_{to}) and L-type Ca²⁺ currents (I_{Ca,L}), patch pipettes were filled with the appropriate internal solutions and bathed with external solutions as previously described (Roman-Campos et al. 2009a). APs were elicited at 1 Hz using short-current square pulses (3-5 ms). I_{to} was elicited by 3 s depolarisation pulses ranging from -40 to +70 mV from a holding potential of -80 mV at increments of 10 mV (15 s interval). I_{Ca,L} was measured at a holding potential of -80 mV, which was increased to -40 mV for 50 ms to inactivate Na⁺ and T-type Ca²⁺ channels. I_{Ca,L} was then measured at different membrane voltages between -40 and 50 mV (300 ms duration and 10 s intervals between test pulses).

Ca²⁺ imaging was performed in Fluo-4 AM (10 μM)-loaded cardiomyocytes that were stimulated at 1 Hz using a Zeiss LSM 510 META confocal microscope equipped with an argon laser (488 nm) and a 63X oil immersion objective in line-scan mode (Roman-Campos et al. 2010). The intracellular Ca²⁺ levels were reported as the F/F₀ ratio, where F is the maximal measured fluorescence and F₀ is the background fluorescence. To fit Ca²⁺ transient decay, we used a mono-exponential function.

All results are expressed as the mean ± standard error of the mean (SEM). For statistical analysis, we used Student's *t* test and *n* represents the number of different cells. *p* < 0.05 was considered statistically significant.

To study the cellular mechanism of cardiomyocyte dysfunction during the chronic phase of Chagas disease, in which *T. cruzi* is no longer observed in the bloodstream (Roman-Campos et al. 2009b), we first measured the transmembrane APs of cardiomyocytes isolated from

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control and infected mice [200 days post-infection (dpi)]. Chagasic cardiomyopathy caused an increase in the time to AP repolarisation (APR). The lengths of time to 90% APR were 27.9 ± 2.2 ms ($n = 13$) and 36.2 ± 2.7 ms ($n = 13$) in control and infected mice, respectively (Fig. 1A, B). The regulation of APR in mouse cardiac myocytes is dependent on I_{to} (Roman-Campos et al. 2010). As shown in Fig. 1C, D, at 200 dpi, cardiomyocytes showed a significant reduction in I_{to} density. The peak outward K^+ current density at +50 mV was 25.9 ± 3.0 pA/pF ($n = 13$) and 14.3 ± 0.5 pA/pF ($n = 4$) in control and infected cardiac myocytes, respectively. In addition to I_{to} , $I_{Ca,L}$ also plays an important role in the control of AP waveform, mainly in the plateau phase. As shown in Fig. 2A, B, $I_{Ca,L}$ was compromised in diseased mice. At 0 mV, the $I_{Ca,L}$ was -7.2 ± 0.6 pA/pF ($n = 8$) and -5.5 ± 0.4 pA/pF ($n = 7$) in control and infected cardiac myocytes, respectively. Furthermore, $I_{Ca,L}$ plays a central role in triggering calcium release from the sarcoplasmic reticulum (SR); thus, reduced $I_{Ca,L}$ could impair calcium release from the SR (Roman-Campos et al. 2010). As shown in Fig. 2C, D, cardiomyocytes isolated from infected mice showed a ~17% reduction in calcium release from the SR compared to control cardiomyocytes. Additionally, calcium reuptake into the SR, which was measured as the calcium transient decay time constant, was slowed by ~39% in myocytes isolated from infected mice (Fig. 2E).

In the present study, at 200 dpi, we showed that chagasic cardiomyopathy led to profound cardiomyocyte dysfunction due to (i) AP prolongation, (ii) reduced tran-

sient outward potassium and L-type calcium currents and (iii) reduced calcium release from the SR.

In previous studies, our and other groups have provided evidence showing that Chagas disease causes the disruption of heart and cardiomyocyte function, primarily due to a sustained inflammatory response, leading to nitric oxide and superoxide anion production (de Carvalho et al. 1992, Machado et al. 2000, Wen et al. 2006, Macao et al. 2007, Sales et al. 2008, Roman-Campos et al. 2009a, 2012). Additionally, it is accepted that sustained and excessive superoxide production is able to downregulate heart and cardiomyocyte function, mainly through the disruption of excitation-contraction coupling (Prosser et al. 2011) and reduction of Kv4.3 expression (Zhou et al. 2006). Nitric oxide is also able to differentially modulate cardiomyocyte function depending on its concentration (Gonzalez et al. 2008). Thus, it is reasonable to postulate that cardiomyocyte dysfunction during the late phase of Chagas disease is due to the excessive production of both nitric oxide (Roman-Campos et al. 2012) and superoxide anion.

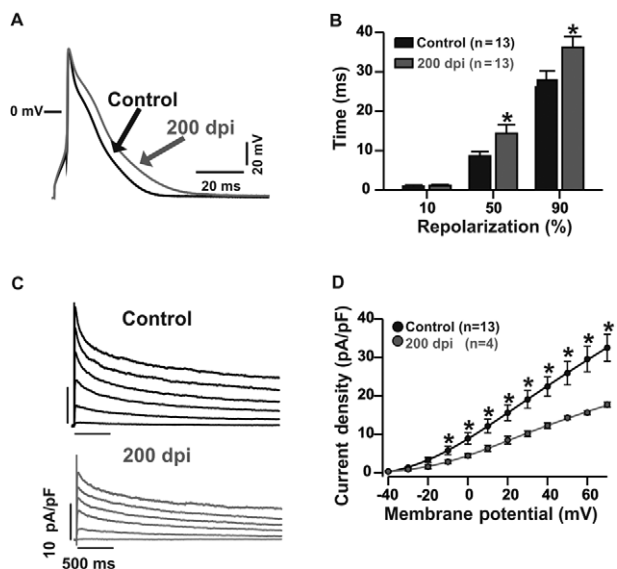


Fig. 1: prolonged action potential (AP) in chagasic cardiomyocyte at 200 days post-infection (dpi). A: representative AP traces from control (black) and chagasic cardiomyocytes (gray); B: AP repolarisation duration at different repolarisation levels; C: reduced transient outward K^+ current (I_{to}) in Chagas disease; D: voltage-dependence of I_{to} plotted as current density (pA/pF) vs. membrane potential (mV) (control: black circles; chagasic cardiomyocytes: gray circles). Continuous lines were obtained by nonlinear regression analysis. Asterisks mean $p < 0.05$. n: number of cells.

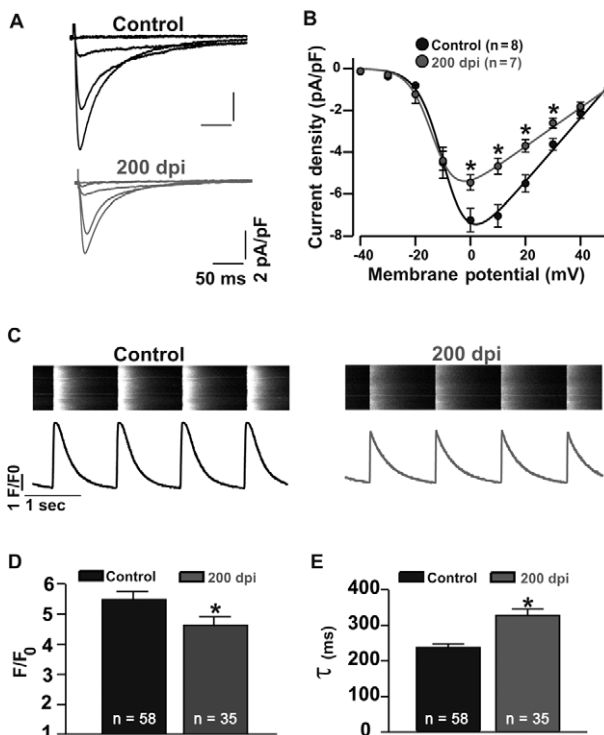


Fig. 2: disrupted excitation-contraction coupling in chagasic cardiomyocytes. A: representative $I_{Ca,L}$ recordings from control (top) and chagasic cardiomyocytes (bottom); B: voltage-dependence of $I_{Ca,L}$ plotted as current density (pA/pF) from -40 to 50 mV (control: black circles; chagasic cardiomyocytes: gray circles); C: representative intracellular global Ca^{2+} transients obtained from electrically stimulated myocytes (top). Ca^{2+} transient profile (bottom); D: significant reduction in peak Ca^{2+} transient amplitude observed in freshly isolated adult chagasic ventricular cardiomyocytes compared to the amplitude of control myocytes; E: time-constant of Ca^{2+} transient decay in chagasic and control cardiomyocytes. Asterisks mean $p < 0.05$. dpi: days post-infection; n: number of cells.

Additionally, we observed reduced calcium release and slowed calcium reuptake into the SR at 200 dpi, both of which are hallmarks of human and mouse models of heart failure (Gwathmey et al. 1987, Lara et al. 2010, Roman-Campos et al. 2010). Thus, our results could explain the reduced heart function observed in the chronic phase of Chagas disease in mice (Jelicks et al. 2002) and may be translated to humans (Marin-Neto et al. 2007). However, additional studies with human cardiac tissue are necessary to confirm this possibility.

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