Mechanical and energetic effects of chronic chagasic patients’ antibodies on rat myocardium

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1Instituto de Investigaciones Cardiológicas, School of Medicine (Universidad de Buenos Aires-Consejo Nacional de Investigaciones Científicas y Técnicas) and 2Biophysical Department, School of Dentistry, Universidad de Buenos Aires, 1122, Buenos Aires, Argentina; and 3Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, 21949-900 Rio de Janeiro, Brazil

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Savio-Galimberti, Eleonora, Patricia Dos Santos Costa, Antonio Carlos Campos de Carvalho, and Jorge Emilio Ponce-Hornos. Mechanical and energetic effects of chronic chagasic patients’ antibodies on rat myocardium. Am. J. Physiol. Heart Circ. Physiol. 287:H1239–H1245, 2004. First published May 20, 2004; 10.1152/ajpheart.01155.2003.—Chagasic (Ch) and nonchagasic (NCh) IgG fraction (20 μg/ml) effects on cardiac performance of adult Wistar rat ventricles were studied with a novel approach applying a microcalorimetric technique. Resting heat (Hr) was significantly decreased by Ch antibodies (ΔHrCH = 4.8 ± 0.9 mW/g). Although the Hr decrease can be associated with diminished activity of the Na+/K+ pump, the magnitude of the effect (25% of control Hr) indicates that additional processes may also be affected. Ch antibodies induced an initial increase in developed pressure (P), which was associated with a decreased contractile economy. However, after 30 min of Ch antibody perfusion, P reached a significantly lower level (ΔPCh = 3.8 ± 1.2 mN/mm2) without changes in active heat per beat (Hr). Consequently, Hr/P ratio increased, indicating that the energetic cost per unit of P was higher. In contrast, P and Hr were both significantly and reversibly decreased by NCh antibodies (ΔPNCCH = 4.4 ± 1.2 mN/mm2; ΔHrNCCH = 9.7 ± 2.2 mJ/g), but Hr/P remained unaffected. According to these data, normal hearts exposed to Ch antibodies present a biphasic mechanical response: 1) an initial period of increased contractility (and decreased global muscle economy) consistent with antibodies with β1-adrenergic activity, such as those used in the present study, and 2) a decrease in P at 30 min of Ch antibody perfusion, which suggests that another Ca2+-related mechanism is compromised. These data contribute to redefine the role of antibody-mediated responses in the pathophysiology of chronic chagasic cardiomyopathy as agents of myocardial failure.

cardiomyopathy; energy metabolism; ventricular function; muscle economy; excitation-contraction coupling

CHAGAS DISEASE is a widespread endemic disease caused by the protozoan Trypanosoma cruzi, which affects many Latin American countries including Argentina and Brazil. The acute phase of the disease, when parasites are present in the circulation, is characterized by a febrile illness, hepatosplenomegaly, lymphadenopathies, and electrocardiographic abnormalities (such as sinus tachycardia, prolongation of atrioventricular interval, and primary T wave changes). During the acute phase patients acquire a positive serology for T. cruzi. At the end of this phase, the number of parasites in circulating blood decreases because of the immune response of the host. The disease then enters into a latent phase in which there are neither clinical signs related to the infection nor parasites in the circulation but persistence of antibodies that react against different structures present in the parasite and the host (10, 28).

About 70–85% of infected people continue in this state, known as the indeterminate form of Chagas disease, for the rest of their lives. However, 15–30% evolve to the chronic phase, years or decades after the primary infection. This chronic phase is characterized by the presence of organ damage, typically cardiomyopathy and/or dysfunction of the gastrointestinal system, resulting in the so-called megasymphies (3).

In Brazil, Chagas disease is the most frequent cause of heart failure (4, 29), and up to 30% of chagasic patients may develop chronic chagasic cardiomyopathy (11). Chronic chagasic cardiomyopathy is mainly caused by damage to myocardial cells, autonomic nervous terminals, and the microcirculation (29). The contribution of autoimmune processes to this pathology was hypothesized in early studies (18). This hypothesis has been supported by a poor correlation between the presence of parasites (either in the myocardium or in the circulating blood) and cardiac damage (32). On the other hand, a good correlation between blood antibody levels against parasites and evolution to a chronic form of cardiac disease has been reported (35) as well as the presence of antibodies that react against cardiac structures during this phase of the disease (28). Borda and colleagues demonstrated the presence of IgGs that interact with β1-adrenergic (6) and muscarinic membrane (15) receptors. The fraction of antibodies that interacts with cardiac β1-adrenergic receptors has been proposed to modulate this receptor, but the consequences of such interaction on heart muscle performance are still controversial (6, 8). Antibodies previously characterized as “β1-adrenergic” by their effect on cardiac electrogenesis have been shown to induce an increase in L-type Ca2+ current in isolated cardiac myocytes by the whole cell patch-clamp technique (L. Barcellos, unpublished observations). Therefore, an effect not only on muscle mechanics (increment in developed pressure (P)) but also on muscle metabolism and energetics can be expected when hearts are exposed to these antibodies.

This work analyzes the effects of IgG (with anti β1-adrenergic properties) obtained from chronic chagasic patients on myocardial performance under resting and active conditions, with special attention to the myocardial contractile economy.

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The application of a combined mechanistic and energetic analysis to study the influence of these antibodies on heart muscle performance is a novel approach in this field. The results show that normal hearts exposed to chronic chagasic patients' antibodies (Ch antibodies) present a biphasic mechanical response that achieves a stable alteration of both global and contractile muscle economies with a consequent increment in the cost of muscle contraction.

**EXPERIMENTAL PROCEDURES**

**Biological preparation.** Wistar rats (250–300 g) were heparinized (1,500 U) and anesthetized with a pentobarbital sodium overdose (administered intraperitoneally). The beating hearts were excised and perfused by the Langendorff method at room temperature (20°C) with control solution. Atria and papillary muscles were dissected from the heart, and a small cut in the septal wall, close to the aorta artery, was made to prevent spontaneous contractions. A latex balloon (connected to a Statham P23-Db pressure transducer) was placed inside the left ventricle, so that P could be measured. The ventricles were mounted in the inner chamber of a calorimeter as previously described (25). Optimal P was functionally established under stimulation (0.16 Hz) by gradual increase of the latex balloon until stable twitch P showed no detectable increase at regular gain. All measurements were performed at the same resting pressure under steady-state conditions and at 25°C. Alterations in resting pressure during the experiment were corrected by inflating or deflating the latex balloon. At the end of each experiment the tissue was removed from the calorimeter, weighed in a preweighed vial, and dried at 110°C to a constant weight so that water content could be calculated. The averaged water content for the present experiments was 0.157 ± 0.01 g (n = 11). Both values are in agreement with previously reported values (5, 19). All of the energetic results included in this study are expressed per gram of dry weight.

The animal procedures used in this investigation conform to the “Guiding Principles for Research Involving Animals and Human Beings” of the American Physiological Society and the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (20).

**Solutions.** The ventricles were perfused at a constant rate (4.5 ml/min) with a control solution containing (in mmol/l) 110 NaCl, 5.5 KCl, 0.5 CaCl₂, 1.3 MgCl₂, 25 NaHCO₃, 1.2 NaH₂PO₄, and 6.0 dextrose. In addition to the control condition, two additional solutions were used: IgG either from nonchagasic patients' sera (NCh) or from chronic chagasic patients' sera (Ch) at a concentration of 20 μg/ml was added to control solution. In all cases, solutions were bubbled with 95% O₂-5% CO₂ (pH 7.4). Protein concentration in sera or IgG fractions was determined by the Bradford method (7).

**Protection of Human Subjects (revised 2001; http://ohrp.osophs.dhhs.gov/humansubjects/guidance/45cfr46.htm).**

**Protocol.** After optimal P was determined the ventricles were kept at rest. Once the equilibration period with control perfusate was elapsed, regular stimulation (0.16 Hz) was resumed. Mechanical and myothermic records during both active and resting steady-state conditions were obtained for control solution, NCh antibody, and/or Ch antibody perfusion. The experimental protocol consisted of a 30-min control period in Krebs solution, a 50-min period of IgG perfusion (with antibody NCh or Ch antibody at concentration of 20 μg/ml in control solution, and a 120-min period of perfusion with control solution (washout period). In all cases the solution changes were done during the active condition.

**Mechanical and heat measurements.** The technique for online measurement of heat production and mechanical activity of isolated heart muscle was previously described in detail (Refs. 24 and 25; E. Savio-Galimberti, unpublished observations). Briefly, the calorimeter was submerged in a constant-temperature bath. The temperature of the calorimeter bath (25°C) was controlled with a cooling-heating bath (±0.003°C) in which the different perfusate solutions were also equilibrated. With this method it was possible to record continuously and simultaneously resting pressure, P, total heat released (Ht), and Hc. The total heat released per beat (Hb) was calculated by the ratio of Ht to the frequency of stimulation, and the active heat per beat (HA) was calculated as the difference of Ht to Hc divided by the frequency of stimulation (0.16 Hz) (24, 25). Global muscle economy was evaluated by the ratio of Hr to maximum P. Contractile muscle economy was evaluated by the ratio of Hr to maximum P (24). For those measurements performed for periods shorter than the integration time of the calorimeter (measurement of Hr associated with the mechanical transient described for Ch experiments) the data were corrected by the following equation (24):

\[
H_r = H_t \cdot \left(1 - A_0 \cdot e^{-t_i} - 8 \cdot \pi^2 \cdot \sum_{i=0}^n A_i \cdot e^{-w\cdot t_i}\right)
\]

where \(A_0 = (\mu \cdot 4 \pi^2 \cdot \beta \cdot \gamma^5 \cdot \tan[\pi \cdot 4 \pi^2 \cdot \beta \cdot \gamma^5], \), \(\mu\) is the cooling rate constant of the calorimeter, \(\beta\) is the diffusion delay constant, \(A_1 = 1/[(2i + 1)^2 \cdot (1 - (2i + 1)^2 \cdot \mu \cdot \gamma^5)], \(i = 2i + 1)^2 \cdot \mu\), \(t\) is time, and \(H_r\) represents a fitted value of the power; \(\beta\) and \(\mu\) were determined as described previously (24).

Both heat and mechanical parameters were recorded in a Grass S7-8 channel recorder, and simultaneously digitized on a desktop computer. Under the experimental conditions used for the present experiments, the time constant of the calorimeter was always <32 s (24). All muscles were initially mounted outside the calorimeter. Reproducible P values throughout the experiment were used as an indication of muscle stability, and this was checked several times (under control perfusion) during the experiment.

**Statistical analysis.** Data are presented as means ± SE, and statistical significance was set at the P < 0.05 level. For paired comparisons the paired t-test was used. ANOVA was used when more than two groups were compared.

**RESULTS**

**Resting condition.** After the equilibration period with control perfusate, the ventricles were kept “quiescent” for ~40 min and Hr was recorded. Because there were no significant differences between the Hr of the control groups for NCh and Ch experiments, an overall averaged Hr was obtained from the pooled data of both control groups. The averaged Hr measured under control conditions was 18.6 ± 2.2 mW/g (n = 11).

**Figure 1** shows the changes in Hr observed after perfusion with solutions containing either NCh or Ch antibodies. The addition
of NCh antibodies to the control perfusate did not significantly affect resting heat production ($\Delta H_{\text{NCh}} = 1.2 \pm 1.3 \text{ mW/g}$). On the other hand, the addition of Ch antibodies to the control solution significantly decreased resting heat production ($\Delta H_{\text{Ch}} = 4.8 \pm 0.9 \text{ mW/g}; P < 0.01, n = 8$).

Active condition. At the beginning of the experiments (and after the resting condition) ventricles were perfused with control solution and stimulated at 0.16 Hz until stable $P$ was reached. Once $P$ remained stable for ~30 min (Fig. 2) the perfusion was changed to either Ch or NCh sera. When perfusion with Ch antibodies was started, an initial increase in $P$ was observed. The maximum $P$ value was reached after ~1 min of the beginning of the perfusion with Ch antibodies (as shown in Figs. 2A and 3A, bottom). The averaged initial increment in $P$ induced by Ch antibodies was $5.4 \pm 0.7 \text{ mN/mm}^2$ ($P < 0.001, n = 8$). In contrast, the ventricles perfused with NCh antibodies showed no significant effects on $P$ immediately after the perfusion was started (Fig. 3A, top).

The initial increment in $P$ ($\Delta P_{\text{Ch}} = 5.4 \pm 0.7 \text{ mN/mm}^2; P < 0.001, n = 8$) observed in the case of the Ch antibody perfusion (Figs. 2B and 3A, bottom) was associated with a transient but significant increase in $H_r$ ($\Delta H_{\text{Ch}} = 57 \pm 7.4 \text{ mJ/g}; P < 0.01, n = 8$; Fig. 3B). Global muscle economy during the transient was evaluated as the ratio of $H_r$ to the corresponding maximum $P$ during the initial period. This ratio was significantly increased during perfusion with Ch antibodies ($\Delta (H_r/P)_{\text{Ch}} = 2.7 \pm 0.7 \text{ mJ.g}^{-1}.\text{mN}^{-1}.\text{mm}^2; P < 0.01, n = 8$; Fig. 3B). These results are in line with the expected activation of the adrenergic receptors by the Ch antibodies.

After 30 min of NCh and Ch antibody perfusion, a new condition was established and $P$ and heat production were...
measured. P was significantly decreased by both NCh
($\Delta P_{NCh} = 4.4 \pm 1.2 \text{ mN/mm}^2; P < 0.05$; Fig. 4A) and Ch
antibodies ($\Delta P_{Ch} = 3.8 \pm 1.2 \text{ mN/mm}^2; P < 0.05, n = 8$; Figs.
2B and 4B). There were no significant differences between the
decreases in P measured in the presence of both antibody
conditions (NCh or Ch: $0.6 \pm 1.2 \text{ mN/mm}^2$).

In three control experiments $H_t$ was measured and showed a
slight but nonsignificant decrease in the presence of NCh
antibodies ($\Delta H_{tNCh} = 18.9 \pm 8.8 \text{ mJ/g}$; Fig. 4A). On the other
hand, $H_t$ significantly decreased in the presence of the Ch
antibodies ($\Delta H_{tCh} = 27.6 \pm 3.8 \text{ mJ/g}; P < 0.01, n = 8$; Fig.
4B). $H_t$ was significantly and reversibly decreased by NCh
antibodies ($\Delta H_{tNCh} = 9.7 \pm 2.2 \text{ mJ/g}$; $P < 0.05$; Fig. 4A). In
contrast, despite the decrease in P induced by the presence of
Ch antibodies in the perfusate, $H_t$ was not significantly af-
fected ($\Delta H_{tCh} = 3.1 \pm 3.6 \text{ mJ/g}; n = 8$; Fig. 4B).

Figure 5A shows typical recordings of isometric P under
control conditions, during the initial increment in P, and at 30
min after the application of Ch antibodies. As mentioned
above, perfusion with Ch antibodies induced an initial increase
in P with a maximum effect reached at 52 ± 8 s after the
perfusion was started (Figs. 2 and 3A, bottom). In addition to
this initial effect, the Ch sera induced a posterior decrease in P
toward a value that averaged $10.3 \pm 2.7 \text{ mN/mm}^2$ at 30 min of
Ch antibody perfusion (Figs. 2B and 4B). Simultaneously with
P changes, maximum rate of relaxation ($-\text{dP/dt}$) was recorded
and its ratio to maximum P was calculated. As shown in Fig.
5B, compared with the value obtained under control conditions,
this ratio significantly increased in the presence of Ch antibod-
ies for both the initial (inotropic) effect $[\Delta ((-\text{dP/dt})/P)_{Ch} = 0.09 \pm 0.02 \text{ s}^{-1}; P < 0.01]$ and the 30-min effect
$[\Delta ((-\text{dP/dt})/P)_{Ch} = 0.16 \pm 0.02 \text{ s}^{-1}; P < 0.001]$.

Global muscle economy, evaluated by the $H_t/P$ ratio, re-
mained unchanged in the presence of NCh antibodies perfusion
$[\Delta (H_t/P)_{NCh} = 1 \pm 0.8 \text{ mJ-g}^{-1} \cdot \text{mm}^{-1} \cdot \text{mm}^2; P > 0.05]$.
On the other hand, despite the decrease in resting heat produc-
tion induced by the presence of Ch antibodies in the perfusate this
$H_t/P$ ratio was significantly increased by the presence of such
antibodies $[\Delta (H_t/P)_{Ch} = 2.5 \pm 1.1 \text{ mJ-g}^{-1} \cdot \text{mm}^{-1} \cdot \text{mm}^2; P < 0.05]$. Similarly to the $H_t/P$ ratio behavior, contractile
muscle economy or $H_{t0}/P$ ratio also remained unchanged under
NCh antibody perfusion $[\Delta (H_{t0}/P)_{NCh} = 0.17 \pm 0.1$
$\text{ mJ-g}^{-1} \cdot \text{mm}^{-1} \cdot \text{mm}^2; P > 0.05]; Fig. 6B$. However, it was significantly
increased by Ch antibody perfusion $[\Delta (H_{t0}/P)_{Ch} = 1.3 \pm 0.2$
$\text{ mJ-g}^{-1} \cdot \text{mm}^{-1} \cdot \text{mm}^2; P < 0.01]; Fig. 6B$. The increment ob-
served in both $H_t/P$ and $H_{t0}/P$ ratios in the presence of Ch
antibodies indicates a higher energy cost per unit of P. It should
also be noted that the increase in both economy ratios induced
by Ch antibodies remained unchanged after a washout with
control solution for up to 120 min (data not shown).

DISCUSSION
The $H_t$ measured in the presence of control perfusate
$(18.6 \pm 2.2 \text{ mW/g}; n = 11)$ is in agreement with the values
reported in the literature ($5, 14$). As shown in Fig. 1, $H_t$ was
affected by the presence of Ch antibodies but it remained

Fig. 5. A: original representative P records obtained from 1 experiment under control perfusate and in the presence of perfusion containing Ch antibodies at 2 different times: immediately after the perfusion with Ch antibodies was started (initial effect; Ch) and after 30-min perfusion with the Ch antibody solution (Ch 30 min). B: ratio between maximum rate of relaxation ($-\text{dP/dt}$) and maximum P ($-\text{dP/dt}/P$) under control perfusate, immediately after the perfusion with Ch antibodies was started (Ch), and after 30-min perfusion with Ch antibody solution (Ch 30 min). #P < 0.001.
The increased $H/P$ ratio described at the beginning of the perfusion with Ch antibodies with β-adrenergic effect is also in agreement with the observed effects of catecholamines (and β₁-adrenergic agonists) on cardiac muscle mechanics and heat production (13, 16, 17). Catecholamines produce an increase in developed tension, which is accompanied by changes in heat production (mainly tension-independent heat), significantly increasing the heat released per unit of developed tension and decreasing the economy of the excitation-contraction process (13, 16, 17).

After the initial positive inotropic effect, $P$ decreased in the presence of Ch antibodies (Figs. 2B and 4B) and, despite the decreased $H$, this fall was accompanied by a decrease in $H'$. Because $H'_c$ remained unchanged (Fig. 4B), the effect on $H'$ was mainly due to the decrease in $H$. As a consequence, both global and contractile muscle economies were significantly affected (Fig. 6). Furthermore, it should be stressed that the effect was not reversed even after a 120-min period of washout with control solution. Therefore, after 30 min of perfusion with Ch antibodies, the combination of a decreased $P$ (Figs. 2B and 4B) and an increased $(\Delta P/\Delta t)/P$ ratio (Fig. 5B) together with the decreased global and contractile muscle economies (Fig. 6, A and B, respectively) suggests that in addition to the initial activation of the β₁-adrenergic mechanism (which would explain the initial positive inotropic effect) other muscle mechanisms are being altered by the antibodies. Because all the experiments were performed under conditions where muscle length was fixed at nearly optimal length and kept fairly unchanged during each experiment (by correcting resting pressure), the diminished $P$ could be related to a decreased Ca²⁺ offer to the myofilaments. This decreased Ca²⁺ offer can be the result of an increased Ca²⁺ removal, which at least in part is suggested by the increased $(\Delta P/\Delta t)/P$ ratio (Fig. 5B). In this regard, Sterin-Borda et al. (30) and Pascual et al. (22) reported that these antibodies would activate the sarcolemmal (SL) Ca²⁺ pump. Because Ca²⁺ removal via the SL Ca²⁺ pump (1 Ca²⁺/1 ATP hydrolyzed) is energetically more expensive than via the sarcotubular (SR) Ca²⁺ pump (2 Ca²⁺/1 ATP hydrolyzed), an activation of the SL Ca²⁺ pump (partially replacing the activity of the SR Ca²⁺ pump) would be in line with the decreased contractile muscle economy observed in the present experiments for the Ch sera.

Another Ca²⁺-removal mechanism that should be considered for a decreased economy is the Na⁺/Ca²⁺ exchanger. Although no previous work has been done with Ch sera on the activity of the Na⁺/Ca²⁺ exchanger, it is of interest to note that the energetic cost of Ca²⁺ removal by the Na⁺/Ca²⁺ exchanger is, like that for the SL Ca²⁺ pump, higher than for the SR Ca²⁺ pump. This is because Ca²⁺ removal via the SL Na⁺/Ca²⁺ exchanger uses 1 ATP per 1 Ca²⁺ removed (because the 1 Ca²⁺/3 Na⁺ stoichiometry of the exchanger requires the use of 1 ATP by the Na⁺/K⁺ pump for the removal of the 3 Na⁺ that entered the cell). Therefore, an activation of the Na⁺/Ca²⁺ exchanger (also partially replacing the activity of the SR Ca²⁺ pump) would lead to a decreased muscle economy.

Another explanation for a decreased Ca²⁺ offer could be the inactivation of the Ca²⁺-releasing processes. Alternatively, this decrement in Ca²⁺ release could result from the activation of another membrane receptor associated with an inhibitory G protein by antibodies present in the total IgG fraction used in

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**Fig. 6.** A: comparison of global muscle economy (evaluated as $H'_c$-to-$P$ ratio) obtained under control perfusate (C), NCh perfusion, and Ch perfusion. B: comparison of contractile muscle economy (evaluated as $H'_c$-to-$P$ ratio) obtained under control perfusate (C), NCh perfusion, and Ch perfusion. As observed, muscle economy (both global and contractile) was only significantly affected by the presence of Ch antibodies in the perfusion media. *$P < 0.01$, **$P < 0.05$.

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unaffected by the presence of NCh antibodies. The magnitude of the decrease (∼25% of $H_c$ under control conditions) indicates that major energy expenditure mechanisms were affected. The major processes involved in resting energy expenditure in heart muscle cells are mitochondrial activity, which includes ATP manufacture and maintenance of the mitochondrial membrane potential (∼30% of basal metabolism), maintenance of the transmembrane Na⁺ gradient by the sarcolemmal Na⁺/K⁺ pump (∼20–25%), maintenance of the transsarcolemmal Ca²⁺ gradient (5%), and resting actomyosin ATPase activity (∼5%) (14, 23, 26). In line with our findings, Pascual’s (21) and Baba’s (2) groups reported that IgG-type antibodies with β₁-adrenergic activity (obtained from chagasic and dilated cardiomyopathic patients) decreased P, release from the spontaneous beating atria and from sarcolemmal preparations and attributed this effect to a decrease in Na⁺/K⁺ pump activity. Even though a diminished activity of the Na⁺/K⁺ pump could be involved in the decrease of the $H_c$ described in the presence of Ch antibodies, the magnitude of the effect on $H_c$ clearly indicates that this is not the only basal metabolism mechanism affected and that additional processes might also be affected.

The initial increase in $P$ observed in the presence of Ch antibodies (Figs. 2B, 3, and 5A) is consistent with the β₁-adrenergic activity proposed for these antibodies in the literature (6). The increment in the $(\Delta P/\Delta t)/P$ ratio observed during this initial response can also be related to a β₁-adrenergic activation (Fig. 5B). The increment in $P$ was associated with a significant increase in $H'_c$ and also with an increased $H'/P$ ratio (Fig. 3B), indicating an increment in the energetic cost per unit of $P$ with the consequent alteration of global muscle economy.
this study. In experiments where ECGs were recorded in the isolated rat heart, the sera used in the present study show a maintained increase in beat rate even after 30 min of perfusion. It is therefore unlikely that the Ch antibodies with β-adrenergic activity induce desensitization or an internalization of the β-receptors. We therefore propose that the observed decrease in P is the result of an effect that is not related to β-adrenergic effect of the Ch sera.

In the presence of an IgG fraction from nonchagasic patients a depressor effect on both mechanical and energetic active parameters was also observed (Fig. 4A), but this effect was fully reversible. Nonetheless, and in contrast with the effect of Ch antibodies, because mechanical and energetic parameters were proportionally decreased, $H'_P/P$ and $H''_P/P$ ratios were not significantly affected by NCh antibodies (Fig. 6, A and B, respectively), indicating that the energy cost per unit of P was preserved.

An increase in the energetic cost of contraction in the heart will certainly aggravate the already reduced mechanical performance of the organ, particularly if this effect is stable as time elapses. The finding that Ch antibodies with known β-adrenergic activity are responsible for this effect is rather unexpected. The initial effect reported in this study is more akin to a β-adrenergic effect, following the classic receptor activated pathways. We speculate, given the long time course of the steady-state effect of the Ch antibodies, that these antibodies may be acting intracellularly, directly interacting with the Ca$^{2+}$ release or uptake systems and/or with the contractile machinery. Internalization of antibodies of different types has been reported, and functional effects for these internalized antibodies have been described in a number of studies (1, 9, 27, 33). The internalization of antibodies is nowadays being studied in a very wide variety of diseases, including classic autoimmune diseases such as systemic lupus erythematosus and systemic sclerosis (27, 28). In the case of these diseases, the presence of antibodies targeting the nucleus or other intracellular organelles could play a central role in the pathogenesis of the altered cellular physiology (1). The implication of such a hypothesis is a new role for the antibodies present in the sera of chronic chagasic patients, totally disassociated from their effect on surface membrane receptors of cardiomyocytes. The possibility that antibodies of this type can be internalized by the cells and interact with intracellular epitopes would help us better understand their role in the altered cellular physiology, and it would also permit us to evaluate the cellular internalization of antibodies as an eventual therapeutic tool in cellular therapy. In this regard it is interesting to note that IgGs with known muscarinic action derived from chronic chagasic patients also decrease P in atrial strips from rat hearts, but this effect cannot be blocked by atropine, the antagonist of the M2 receptor (C. Leite, unpublished observations).

The decreased energy expenditure observed in the resting, nonstimulated heart, along with the increased energy cost per unit of P in the presence of Ch antibodies, is an entirely novel finding and may have important implications in the pathogenesis of Chagas disease. Clinical evolution of chronic chagasic cardiomyopathy, from electrical to mechanical dysfunction, leads inexorably to heart failure. An antibody-mediated mechanism that increases the energetic cost of contraction undoubtedly could contribute to the failing state of the heart in chronic chagasic cardiomyopathy. The approach used in this work for studying this condition is unique in the literature, and it points out the utility that an energetic study can represent in the understanding of disease states.

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