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# MODIFICATION OF SARCOLEMMAL ENZYMES BY CHAGASIC IgG AND ITS EFFECT ON CARDIAC CONTRACTILITY

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**Abstract**—It has been shown that sera from chagasic patients contain an antibody which binds to β-adrenoceptors of myocardium and modulates their activity. Chagasic IgG triggered a marked stimulation of myocardial contractility with an increase in intramyocardial cyclic AMP and inhibition of $(Na^+ + K^+)$-ATPase activity. Both the mechanical and enzymatic effects of the IgG could be prevented by β-adrenoceptor blockade or after the absorption of chagasic IgG with turkey red blood cells. In contrast, guinea pig red blood cells were unable to remove the β-reactivity of chagasic IgG. These findings suggest that the IgG from chagasic patients increases myocardial contractility by behaving as a β-agonist. This effect is likely related to stimulation of the adenylate cyclase coupled to the cardiac β-adrenoceptor.

Among the immunological factors suspected of involvement in the pathophysiological mechanism of chronic Chagas' heart disease, there is a certain component(s) of IgG fraction from chagasic sera. We have already reported the existence in chagasic patients of circulating IgG that binds and modulates the cardiac β-adrenoceptor [1].

Sera from chagasic patients simulate a partial β-agonist by increasing the tension and contraction frequency of rat-atria preparations and, on the other hand, diminishing reactivity to exogenous norepinephrine. These observations point to an interaction between adrenoceptors and IgG purified from the reactive sera [2, 3]. Moreover, chagasic IgG inhibits the binding of [$^3$H]dihydroalprenolol ((−)-[$^3$H]DHA) to the β-adrenoceptor of purified myocardial membranes by acting as a non-competitive inhibitor. The binding of chagasic IgG with the β-adrenoceptor is lost after absorptions with turkey red blood cells, a cell rich in $\beta_1$-adrenoceptors [1]. The specific pharmacological blockade of the cardiac $\beta_1$-adrenoceptors also inhibits the biological effect of chagasic IgG.

Previous work in this laboratory [4, 5] has shown that sera from chagasic patients modify the inotropic and arrhythmogenic effect of ouabain in isolated rat atria. We found that the normal action of ouabain is restored after β-adrenoceptor blockade. This suggests an adrenergic participation in the response to ouabain which is capable of modification by chagasic sera. Antagonism between the inotropic actions of catecholamines and ouabain has been documented previously [4, 6, 7].

In this study we relate the contractile effect of anti-β-adrenoceptor IgG from chagasic sera with enzyme activities associated with the myocardial cell membrane, namely adenylate cyclase and $(Na^+ + K^+)$-ATPase. It will be shown that chagasic IgG triggered a marked stimulation of myocardial contractility with a parallel increase in intramyocardial cyclic AMP. Furthermore, chagasic IgG appeared to exert an inhibitory effect on sarcolemmal $(Na^+ + K^+)$-ATPase activity. Both the mechanical and enzymatic effects of the IgG were prevented after β-adrenoceptor blockade or after the absorption of chagasic IgG with turkey red blood cells.

## METHODS

*Serum selection: chagasic patients and controls.* Sera were obtained from eight asymptomatic *Trypanosome cruzi* infected individuals, residing at the time in metropolitan Buenos Aires, and from five normal non-infected individuals. Chagas' serology was studied by three standard serological reactions against *T. cruzi*: complement fixation, passive haemoglutination, and immunofluorescence [8]. Endocardium-blood vessels-interstitium (EVI) reactivity was assayed by indirect immunofluorescence with fluorescein-labeled rabbit F $(ab')_2$ anti-human IgG (Cappel Lab., Cochranville, PA, U.S.A.) [2]. Chronic Chagas' heart disease patients were not included in this study to avoid interference with medications.

*Purification of human IgG.* IgG was isolated from sera of eight EVI positive chagasic patients and five normal human sera by precipitation with 40% ammonium sulfate and chromatography with DEAE-cellulose (Bio-Rad, Richmond, CA, U.S.A.) balanced with 0.005 M (pH 8) phosphate buffer. The eluted IgG fractions were concentrated and dialyzed against phosphate buffer solution (PBS), showing one line of precipitation, corresponding to IgG with polyvalent antisera. The

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final IgG concentration was determined by radial immunodiffusion assay.

*Absorption procedures.* Guinea pig and turkey red blood cells (GP-RBC or T-RBC) were washed three times with PBS and incubated with heat-inactivated normal and chagasic serum in a proportion of 1 ml serum/3 ml packed RBC for 1 hr at 37° and 1 hr at 4°. After absorption, EVI positive chagasic sera were tested by indirect immunofluorescence on heart and skeletal muscle reactions [8], to determine that the absorption with GP-RBC had totally removed the EVI reactivity, and the absorption with T-RBC had not modified the EVI pattern, or even the sera titres. These different absorptions with GP- and T-RBC were performed due to the fact that GP-RBC are very rich in EVI antigen [8, 9] and lack $\beta_1$-adrenoceptors, whereas T-RBC are very rich in the latter [10]. IgG, referred to in Results as absorbed with GP-RBC and T-RBC, was obtained from absorbed sera and further controlled by indirect immunofluorescence over myocardium and skeletal muscle.

*Isolated rat atrial preparations.* Male albino rats of the Wistar strain were killed by decapitation. The atria were separated from the ventricles, carefully dissected, attached to a glass holder, and immersed in a tissue chamber containing a modified Krebs–Ringer–bicarbonate (KRB) solution, gassed with 5% $CO_2$ in $O_2$, maintained at pH 7.4 and 30°. The ionic composition of KRB was reported previously [2]. A constant resting tension of 750 mg was applied to the atria, and the activity of spontaneous beating atria was assessed, recording the maximum rate of isometric force development (dF/dt) above the externally applied resting tension. The atria were allowed to function for 1 hr. Records were then made, and the values of the initial controls were considered as 100%. The control value of dF/dt at the end of equilibrium and before the addition of IgG was 7.8 ± 1.2 g/sec (N = 10).

*Inorganic phosphate ($P_i$) release by beating atria.* In this method, the inorganic phosphate ($P_i$) release was determined in KRB in which atrial preparations were beating and subjected to the same conditions described for mechanical measurements. Thus, atria were immersed under preload tension in the same tissue chamber filled with 2.5 ml of KRB solution equilibrated at 37° and pH 7.4 with 5% $CO_2$ in $O_2$. The volume of incubation solution was constant throughout the experiments and was constituted as follows (mmoles/l): $Na^+$, 145; $K^+$, 6.02; $Mg^{2+}$, 1.33; $Cl^-$, 126; $HCO_3^-$, 25.3; $SO_2^{2-}$, 1.33; $PO_4^{3-}$, 1.20; and $Ca^{2+}$ 1.22. The concentration of $P_i$ in this solution was 125 mmoles/l. No exogenous ATP was added. Atria beating spontaneously were incubated in this solution during 45 or 60 min, and 100 μl of buffer was taken every 15 min. To each sample, 1000 μl of ferrous sulfate-sodium-molybdate reagent was added. $P_i$ was determined by the method of Taussky and Shorr [11]. The intensity of the color was determined in a Zeiss Specktro photometer PM 2 DL at 750 nm. A blue color developed maximally within 1 min and was stable for at least 2 hr. There was a linear relationship between the colorimetric reading and the concentration of phosphorus. Absolute values of $P_i$ were expressed as nmoles of $P_i$ released in incubation solution per 100 mg of tissue dry weight. The mean value of atria dry weight was 20 ± 2 mg (N = 25). In order to know the metabolic state of the atria, the ATP/ADP ratio and the creatine phosphate (CP) content were measured. Atria were frozen with liquid nitrogen at 0, 15, 30 and 45 min after atria were beating in KRB. The tissue was pulverized and the powder was extracted in 2 vol. of cold 6% perchloric acid. The neutralized extract was used for analysis of adenine nucleotides and CP by standard enzymatic assays [12]. The ATP/ADP radio (5.52) and the CP (22.8 ± 5.6) in μmoles/g dry wt were unchanged during the above-mentioned periods.

*ATPase activity in sarcolemmal fraction.* Sarcolemma was prepared by the hypotonic shock-LiBr treatment method as described by McNamara *et al.* [13]. Briefly, hearts were excised and placed in ice-cold 10 mM Tris–HCl buffer containing 1 mM EDTA, pH 7.4. The ventricles were washed in the buffer, cut into small pieces, and homogenized by a Polytron PT-20 at a setting of 3 for 15 sec in 10 vol. of 10 mM Tris–HCl, pH 7.4, containing 1 mM EDTA. The homogenate was filtered through cheesecloth and centrifuged at 1000 *g* for 10 min. The sediment was suspended in 20–25 vol. of 10 mM Tris–HCl buffer, pH 7.4, stirred in the cold-room for 30 min, and centrifuged at 1000 *g* for 10 min. This process was repeated two more times, first by suspending the sediment in 10 mM Tris–HCl buffer, pH 8.0, and then in the same buffer but at pH 7.4. The sediment was suspended in 20–25 vol. of 10 mM Tris–HCl, pH 7.4, extracted with 0.4 M LiBr for 30 min, and centrifuged at 1000 *g* for 10 min. The sediment was again suspended in 10 mM Tris–HCl buffer, pH 7.4, stirred for 10 min, and centrifuged at 1000 *g* for 10 min. The sediment was further extracted with 0.6 M KCl, stirred, centrifuged, and once again washed in 10 mM Tris–HCl buffer, pH 7.4. The sarcolemma fraction thus obtained was suspended in 10 mM Tris–HCl buffer, pH 7.0, and used within 1–2 hr. Membrane suspensions (1 to 1.5 mg/ml protein) were incubated with a $5 \times 10^{-7}$ M dilution of normal and chagasic IgG for 1 hr at 4° in 1 mM Tris–HCl buffer, pH 7.4. These membranes were then washed twice by centrifugation and resuspended in the same volume. For ATP hydrolyzing activity, an 80–100 μg sample of membrane protein per ml in a medium containing 50 mM Tris–HCl (pH 7.5), 100 mM NaCl, 10 mM KCl, 4 mM $MgCl_2$, and 1 mM EDTA in the presence or the absence of 2 mM ouabain was incubated for 3 min at 37°, and the reaction was then started with 4 mM Tris–ATP and stopped 10 min later by the addition of 12% ice-cold trichloroacetic acid. The $P_i$ in the supernatant fraction was analyzed according to the method of Taussky and Shorr [11]. Protein concentration was measured by the method of Lowry *et al.* [14]. The difference of the activity in the absence and presence of ouabain is referred to as $Na^+$-$K^+$-stimulated $Mg^{2+}$-dependent, ouabain-sensitive ATPase ($Na^+ + K^+$-ATPase). The ($Na^+ + K^+$)-ATPase activity is expressed as micromoles of $P_i$ per milligram of protein per hour (μmoles $P_i$/mg/hr). The $Mg^{2+}$-ATPase and $Ca^{2+}$-ATPase activities were determined according to Sulakhe and Dhalla [15] by incubating the membranes in a medium containing 50 mM Tris-

Table 1. Characterization of myocardial sarcolemmal fractions

| | Enzyme activities | | | |
|---|---|---|---|---|
| | $(Na^+ + K^+)$-ATPase ($\mu$moles $P_i$/mg/hr) | $Mg^{2+}$-ATPase ($\mu$moles $P_i$/mg/hr) | $Ca^{2+}$-ATPase ($\mu$moles $P_i$/mg/hr) | 5′-Nucleotidase ($\mu$moles $P_i$/mg/10 min) |
| Homogenate | 1.6 ± 0.1 | 16.6 ± 1.6 | 17.3 ± 2.2 | 0.16 ± 0.03 |
| Sarcolemma | 6.8 ± 0.2 | 13.1 ± 1.1 | 30.3 ± 1.2 | 0.82 ± 0.04 |

HCl (pH 7.5), 1 mM EDTA, and 4 mM ATP with or without 4 mM $MgCl_2$ or $CaCl_2$. 5′-Nucleotidase activity was measured according to Erecinska *et al.* [16]. Relative activities of the "marker" enzymes indicate the degree of purity of the sarcolemmal fraction (Table 1).

*Cyclic AMP assay.* Atria beating spontaneously were incubated for 1, 5, 10, 15 and 20 min at 30° with normal IgG ($5 \times 10^{-7}$ M), chagasic IgG ($5 \times 10^{-7}$ M) or isoproterenol ($1 \times 10^{-9}$ M) with or without propranolol ($1 \times 10^{-7}$ M). Controls with no IgG added were carried similarly. After a 30-min incubation, tissues were homogenized in 1.0 ml of 6% ice-cold trichloroacetic acid (TCA) and centrifuged at 2500 *g* for 15 min at 4°. Proteins were determined by the method of Lowry *et al.* [14] after dissolving them in 1 ml of 1 N NaOH in boiling water. The TCA supernatant fractions were extracted with 4 ml of water-saturated-ethyl ether, three times. The ether phase was discarded, and the aqueous phase was heated at 56° to remove the ether and evaporated to dryness under a stream of nitrogen gas. Cyclic AMP in the residue was dissolved in 300 $\mu$l of 0.05 M sodium acetate buffer, pH 6.2. Aliquots of 100 $\mu$l were taken for nucleotide determination using a radioimmunoassay (Cyclic AMP-[$^{125}$I]RIA KIT, New England Nuclear).

*Drugs.* Freshly prepared stock solutions of the following drugs were used: (−)-propranolol (Ayerst Lab., New York, NY, U.S.A.); isoproterenol, dibutyryl cAMP, theophylline and ouabain octahydrate (Sigma Chemical Co., St. Louis, MO, U.S.A.). An aliquot of each stock solution was added to the tissue bath to achieve the final concentrations mentioned in the text.

*Statistics.* The experimental results were analyzed by Student's *t*-test. Differences in means were considered significant if P was equal to or less than 0.05.

## RESULTS

*Effect of chagasic IgG on intracellular cAMP level.* As seen in Table 2, chagasic IgG increased the intracellular levels of cAMP above basal value. The increment induced by $5 \times 10^{-7}$ M chagasic IgG was similar to that observed with $1 \times 10^{-9}$ M isoproterenol. Propranolol ($10^{-7}$ M) completely inhibited the stimulatory effect of chagasic IgG on intracellular cAMP levels. When chagasic IgG was previously absorbed with GP-RBC, the stimulatory effects were enhanced, but when the IgG was absorbed with T-RBC the effect was abolished (Fig. 1). Normal IgG samples treated as chagasic failed to increase the intracellular cAMP levels above basal value (Table 2 and Fig. 1).

Table 2. Responsiveness of cyclic AMP levels of rat heart membranes

| Additions | cAMP (pmoles/mg wet wt) |
|---|---|
| None | 0.51 ± 0.05 |
| Isoproterenol ($10^{-9}$ M) | 1.37 ± 0.10* |
| Chagasic IgG ($5 \times 10^{-7}$ M) | 1.25 ± 0.09* |
| Chagasic IgG + propranolol ($10^{-7}$ M) | 0.42 ± 0.04 |
| Normal IgG ($5 \times 10^{-7}$ M) | 0.49 ± 0.06 |

Values are means ± S.E.M. of eight separate experiments performed in duplicate. Values were measured in atrial homogenates after 3 min of reaction with additions indicated. Before that, atria were stabilizated during 30 min in KRB with or without propranolol.

* $P < 0.0005$ between none versus isoproterenol or chagasic IgG.

Figure 2 correlates the contractile effect of chagasic IgG with cAMP levels increased as a function of time. It can be seen that, during the first 5 min, chagasic IgG increased cAMP levels, whereas from 5 to 20 min they decreased and dF/dt increased. As described for the cAMP effect, the contractile effect of the antibody was blunted by propranolol (data not shown), confirming that the stimulatory effects of chagasic IgG were mediated predominantly by a $\beta$-adrenergic mechanism.

*Effect of chagasic IgG on "$P_i$ release".* The ability of the chagasic IgG to induce changes in the $P_i$ release was assayed. As shown in Fig. 3, chagasic IgG inhibited the $P_i$ release by the atria beating spontaneously in KRB in comparison with normal IgG. To ascertain whether the inhibition of $P_i$ release by chagasic IgG was triggered through a $\beta$-adrenoceptor mechanism, the propranolol effect was explored. As seen in Fig. 3, the incubation of the atria with propranolol ($10^{-7}$ M) prevented the inhibitory action of chagasic IgG on the $P_i$ release. Normal IgG was inactive in the system. Values obtained with normal IgG with or without propranolol were similar to those of KRB alone, with or without $\beta$-blocker (Table 3).

As shown in Fig. 4, when chagasic IgG was previously absorbed with GP-RBC, the inhibitory effect upon the $P_i$ release was enhanced in comparison with chagasic IgG without absorption. In contrast, when the same IgG was absorbed with T-RBC, the effect was prevented, values proving similar to those of normal IgG (Fig. 3). Table 4 compares the inhibitory effect of chagasic IgG on the $P_i$ release with other interventions capable of increasing intracellular

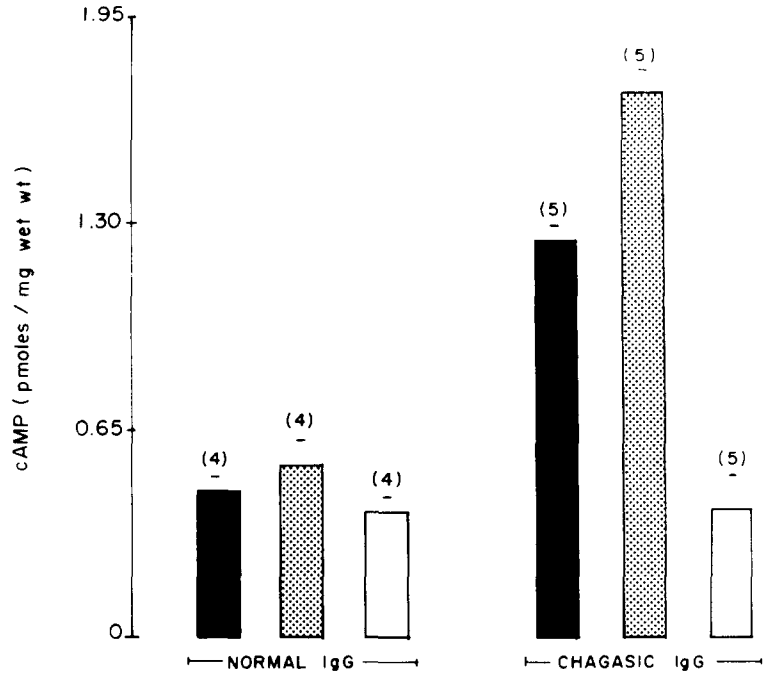


Fig. 1. Effects of chagasic and normal IgG before (■) and after absorption with GP-RBC (▨) or T-RBC (□) on cAMP. Atria were incubated for 30 min in KRB. cAMP was measured after 3 min of IgG additions. Values are mean ± S.E.M. from eight patients; experiments were performed in duplicate.

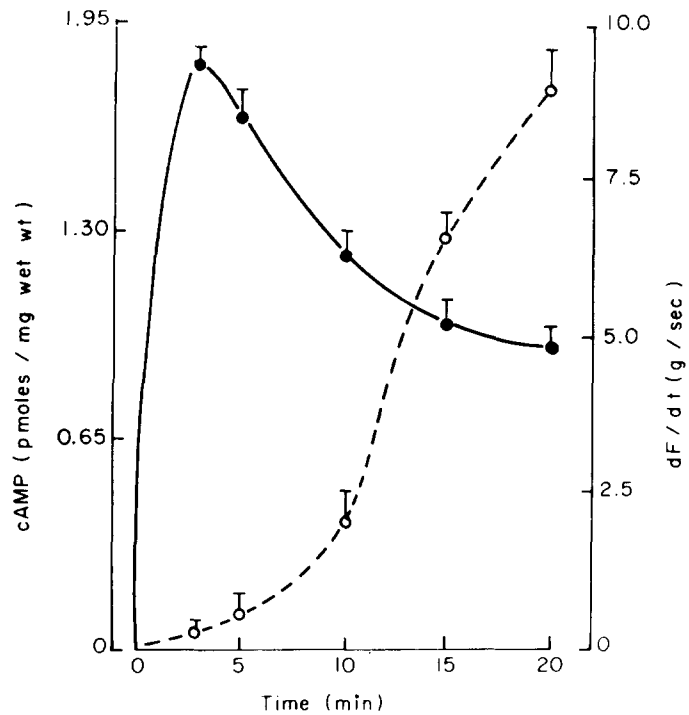


Fig. 2. Time course of contractile (○---○) and cyclic AMP (●—●) effects of chagasic IgG absorbed with GP-RBC. Values are means ± S.E.M. from five different patients. Each point is the value at a given minute of perfusion with chagasic IgG, obtained after a 30-min stabilization period.

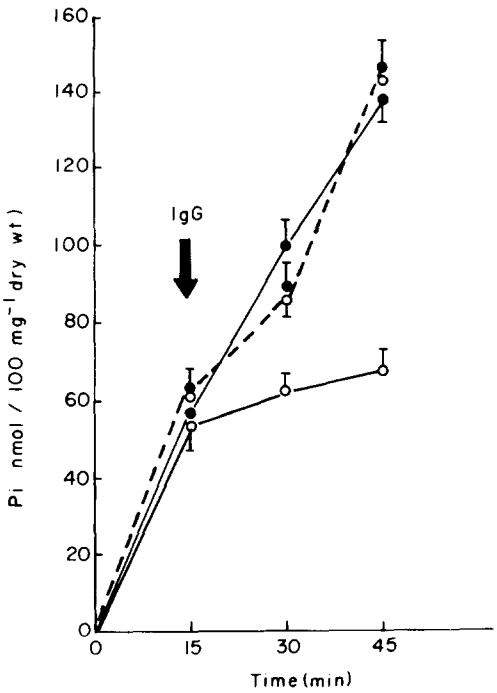


Fig. 3. Time course of $P_i$ release by isolated rat atria beating spontaneously. Key: effect of chagasic (○—○) and normal (●—●) IgG; influence of propranolol ($10^{-7}$ M) on the effect of chagasic (○---○) and normal (●---●) IgG. The arrow indicates IgG additions at 15 min. Values are means ± S.E.M. from eight chagasic and five normal patients.

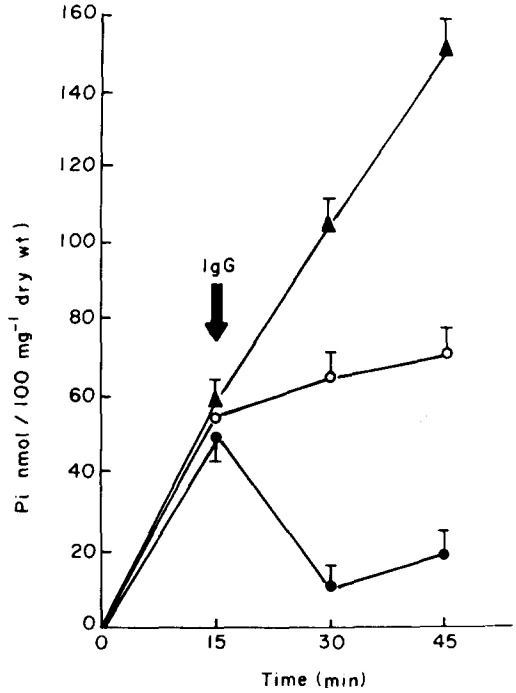


Fig. 4. Time course of $P_i$ release by isolated rat atria beating spontaneously in the presence of chagasic IgG without absorption (○—○) or absorbed with T-RBC (▲—▲) or GP-RBC (●—●). Other details are as given for Fig. 3. Values are ± S.E.M. of eight different patients.

cAMP levels. The inhibitory effect was shared by isoproterenol, dibutyryl cAMP and theophylline.

It should be stressed that the release of $P_i$ by isolated rat atria beating spontaneously in KRB increased with time. It appeared to be correlated with $(Na^+ + K^+)$-ATPase activity, since it was stimulated at high potassium concentration (30 mmoles/l) and inhibited at very low potassium concentration (1.2 mmoles/l). Moreover, the $P_i$ release was unaffected by lack of $Mg^{2+}$ in the incubation medium. Ouabain, at concentrations known to inhibit the enzyme, also inhibited the release of $P_i$ (Table 5).

Table 3. Values of $P_i$ release by isolated rat atria beating with or without propranolol

| Incubation of atria with | $P_i$ release (nmoles/100 mg dry wt) at 45 min |
|---|---|
| KRB alone | 136.2 ± 5 |
| KRB + propranolol | 135.8 ± 4 |
| KRB + normal IgG | 140.1 ± 4 |
| KRB + normal IgG + propranolol | 145.2 ± 6 |

Normal IgG ($5 \times 10^{-7}$ M) and propranolol ($1 \times 10^{-7}$ M) were added together at 0 time. Values are means ± S.E.M.; five different normal IgG were measured in duplicate.

Table 6 compares the inhibitory effect of chagasic IgG with isoproterenol on $(Na^+ + K^+)$-ATPase activity in isolated membranes. It can be seen that chagasic IgG, as well as isoproterenol, inhibited the sarcolemmal $(Na^+ + K^+)$-ATPase activity, whereas normal IgG was inactive in the system.

## DISCUSSION

In this paper we demonstrate that a certain component(s) of the IgG fraction from chagasic sera is

Table 4. Comparative effects of different drugs and chagasic IgG on $P_i$ release by isolated rat atria

| Additions | $P_i$ release (% of inhibition) |
|---|---|
| Chagasic IgG ($5 \times 10^{-7}$ M) | 52.2 ± 7 |
| Isoproterenol ($5 \times 10^{-9}$ M) | 51.2 ± 6 |
| Dibutyryl cAMP ($1 \times 10^{-7}$ M) | 49.2 ± 3 |
| Theophylline ($5 \times 10^{-5}$ M) | 42.3 ± 4 |

Values (mean ± S.E.M.) are expressed as percent of changes against controls taken 45 min before additions. There were eight experiments in each group. Basal values: control: 140.2 ± 5; chagasic IgG: 66.9 ± 8.9; isoproterenol: 68.3 ± 8.0; dibutyryl cAMP: 71.7 ± 4.3 and theophylline: 80.8 ± 7.6 nmoles of $P_i$ released per 100 mg of tissue dry weight.

Table 5. Characterization of $P_i$ release by isolated rat-atria preparation beating spontaneously in KRB

| Additions | $P_i$ release (nmoles/100 mg tissue dry wt) | | |
|---|---|---|---|
| | 15 min | 30 min | 45 min |
| None | 62.1 ± 2 | 89.4 ± 4 | 140.5 ± 5 |
| 30 mM $K^+$ | 124.1 ± 3 | 130.7 ± 4 | 159.2 ± 4 |
| 1.2 mM $K^+$ | 40.7 ± 1 | 52.4 ± 3 | 97.2 ± 2 |
| 0 $Mg^{2+}$ | 60.8 ± 3 | 95.4 ± 4 | 140.6 ± 5 |
| Ouabain ($2 \times 10^{-4}$ M) | 30.2 ± 2 | 33.5 ± 3 | 43.2 ± 3 |

Values are means ± S.E.M.; there were 7 experiments in each group. Values in μmoles/g dry tissue obtained with the enzymatic method for ADP (3.6 ± 0.43), ATP (22.4 ± 1.98) and creatine phosphate (22.3 ± 2.70) did not change in atria perfused during 15, 30 and 45 min.

able to mimic the biological effect of catecholamines, triggering a marked stimulation of myocardial contractility. In previous work we have documented the existence of a circulating IgG in chagasic patients which binds with $\beta_1$-adrenoceptors of the heart and which is able to inhibit the binding of (−)-[$^3$H]DHA to the β-adrenergic receptor of purified cardiac membranes. The specific antibody for the $\beta_1$-adrenoceptor is independent of other tissue reactive antibodies, such as the EVI system, and has a significant clinical specificity to heart disease [1].

In this paper the adrenergic participation in the effect of chagasic IgG is strongly supported. Chagasic IgG from the same patients increased cAMP levels and atrial contractility. Both stimulatory actions could be blocked by specific β-adrenoceptor antagonist. This suggests that the mechanism whereby the chagasic IgG may trigger a β-adrenergic biological effect is through a direct interaction between the IgG and the membrane-bound-receptor–cyclase complex. Furthermore, the responses in biological assays and adenylate cyclase activity could be diminished significantly by absorption with T-RBC, a cell rich in $\beta_1$-adrenoreceptors, but devoid of EVI antigen.

Besides cAMP stimulation, other enzymes could be affected by chagasic IgG, notably the ATPases. Chagasic IgG inhibited the $P_i$ release by atria beating spontaneously in KRB. The $P_i$ release from the preparation appeared to be correlated with manipulations which either stimulate or inhibit the ($Na^+ + K^+$)-ATPase, such as ouabain and different potassium concentrations. This is in agreement with Medina and Illingworth [17] who reported an alteration of [$^{32}$P]$P_i$ efflux in rat perfused heart by ouabain, adrenaline and dibutyryl cyclic AMP. Furthermore, β-agonist and chagasic IgG also inhibit $P_i$ release, suggesting that the mechanism by which they alter $P_i$ release may involve inhibition of the ($Na^+ + K^+$)-ATPase.

The specific pharmacological blockade of cardiac β-adrenoceptors restored the $P_i$ release. Moreover, the fact that after the absorption of the IgG with T-RBC the inhibitory action of chagasic IgG on the $P_i$ release was prevented provides evidence that this effect is associated with the β-adrenergic mechanism.

The fact that dibutyryl cyclic AMP, theophylline and isoproterenol also inhibit the $P_i$ release is consistent with the proposition that an increase of cAMP level inhibits the ($Na^+ + K^+$)-ATPase enzyme.

This finding agrees with the results of Limas *et al.* [18], who described an inhibitory action of 3′,5′-cAMP and adrenaline on the ($Na^+ + K^+$)-ATPase of myocardial sarcolemma as well as by the data in this paper showing that both isoproterenol and chagasic IgG inhibit ($Na^+ + K^+$)-ATPase activity in an *in vitro* sarcolemmal preparation. The latter observation is, however, difficult to explain without documenting the presence of an active cyclic AMP dependent effector system under the conditions of our ($Na^+ + K^+$)-ATPase assay. One might even speculate about a more direct mechanism of β-adrenergic ($Na^+ + K^+$)-ATPase interaction though a mechanism of this type has yet to be documented.

On the available evidence it is tempting to speculate that these findings may explain the fact that the chagasic patient behaves as a natural β-blocked responder [19, 20]. Furthermore, it is possible that inhibition of the ($Na^+ + K^+$)-ATPase may be involved in the genesis of cardiac arrhythmias observed in Chagas' heart disease.

Table 6. Inhibitory effect of chagasic IgG on the ($Na^+ + K^+$)-ATPase of sarcolemmal fraction—Comparison with isoproterenol and normal IgG

| Additions | ($Na^+ + K^+$)-ATPase activity | |
|---|---|---|
| | (μmoles $P_i$/mg/hr)* | % of Changes† |
| None | 6.8 ± 0.2 | — |
| Normal IgG ($5 \times 10^{-7}$ M) | 6.9 ± 0.3 | +1.5 |
| Chagasic IgG ($5 \times 10^{-7}$ M) | 4.8 ± 0.1‡ | −29.4 |
| Isoproterenol ($1 \times 10^{-4}$ M) | 4.6 ± 0.1‡ | −32.3 |

* Values are means ± S.E.M.; N = 7. One unit represents the amount of enzyme that hydrolyzes 1 μmole of ATP per mg of protein per hr. N = number of ventricular sarcolemmal preparations.

† Values represent percent of variation from control (no addition) taken as 100%.

‡ $P < 0.0005$ between normal IgG versus chagasic IgG or isoproterenol.

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## REFERENCES

1. E. Borda, J. Pascual, P. Cossio, M. de Vega, R. Arana and L. Steri-Borda, *Clin. expl Immun.* **57**, 679 (1984).
2. L. Sterin-Borda, P. Cossio, M. Gimeno, C. Diez, R. Languens, P. Cabeza-Mecker and R. Arana, *Cardiovas. Res.* **10**, 613 (1976).
3. L. Sterin-Borda, S. Fink, C. Diez, P. Cossio and M. M. Bracco, *Clin. expl Immun.* **50**, 534 (1982).
4. L. Sterin-Bora, L. Canga, P. Cossio, C. Diez, R. Arana and A. L. Gimeno, *Archs int. Pharmacodyn. Ther.* **250**, 93 (1981).
5. L. Sterin-Borda, L. Canga, E. Borda, P. Cossio, C. Diez, R. Arana and A. L. Gimeno, *Eur. J. Pharmac.* **69**, 1 (1981).
6. G. A. Seifen, *Br. J. Pharmac.* **51**, 481 (1974).
7. C. Mendez, J. Acevez and R. Mendez, *J. Pharmac. exp. Ther.* **131**, 191 (1961).
8. P. Cossio, A. Szarfman, E. Kreutzer, B. Candiolo and R. Arana, *Circulation* **49**, 12 (1974).
9. E. L. Khoury, C. Diez, P. Cossio and R. Arana, *Clin. Immun. Immunopath.* **27**, 283 (1983).
10. A. B. Schreiber, P. Oliver-Couraud, C. Andre, B. Vray and A. Donny-Strosberg, *Proc. natn. Acad. Sci. U.S.A.* **77**, 7385 (1980).
11. H. H. Taussky and E. Shorr, *J. biol. Chem.* **202**, 675 (1953).
12. H. U. Bergmeyer, in *Methods of Enzymatic Analysis* (Ed. H. U. Bergmeyer), p. 559. Academic Press, New York (1963).
13. D. B. McNamara, P. V. Sulakhe, J. N. Singh and N. S. Dhalla, *J. Biochem., Tokyo* **75**, 795 (1974).
14. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
15. P. V. Sulakhe and N. S. Dhalla, *Life Sci.* **10**, 185 (1971).
16. M. Erecinska, M. Sierakowska and D. Shugar, *Eur. J. Biochem.* **11**, 465 (1969).
17. G. Medina and J. A. Illingworth, *Biochem. J.* **224**, 153 (1984).
18. C. J. Limas, A. V. Notargiacomo and J. N. Cohn, *Cardiovas. Res.* **7**, 477 (1973).
19. T. F. Caeiro, H. A. Palmero and D. Iosa, *Medicina, Buenos Aires* **40**, 27 (1980).
20. H. A. Palmero, T. F. Caeiro and D. J. Iosa, *Am. Heart J.* **97**, 38 (1979).