Differential Profile and Biochemical Effects of Antiautonomic Membrane Receptor Antibodies in Ventricular Arrhythmias and Sinus Node Dysfunction

Pablo A. Chiale, MD; Inés Ferrari, PhD; Evelina Mahler, MS; Marina A. Vallazza, MD; Marcelo V. Elizari, MD; Mauricio B. Rosenbaum, MD; Mariano J. Levin, PhD

Background—The relationship between anti-β-adrenergic (anti-βR) and anti-M 2 -cholinergic (anti-M2R) receptor antibodies (Abs) and cardiac arrhythmias and their biochemical effects have not been systematically investigated.

Methods and Results—We studied 41 patients, 28 with ventricular arrhythmias (primary or due to Chagas’ heart disease or idiopathic dilated cardiomyopathy; group I), 13 with sinus node dysfunction (primary or caused by Chagas’ heart disease; group II), and 10 healthy controls (group III). The chronotropic effects of the IgG and immunopurified anti-β1R Abs or anti-M2R Abs were assessed on cultured cardiomyocytes before and after exposure to atropine and propranolol. The biochemical effects of the IgG from 9 patients from group I, 6 from group II, and 6 controls were evaluated on COS7 cells transfected with genes encoding for β1, β2-adrenergic receptors (cAMP increment) or M 2 -cholinergic receptors (phosphatidylinositol increment). The IgG from group I patients exerted a positive chronotropic action, with a high prevalence of anti-β1R Abs (75%) and low prevalence of anti-M2R Abs (10.7%) and induced a clear-cut and long-lasting increment in cAMP. The IgG from group II patients depressed chronotropism, with a high prevalence of anti-M2R Abs (76.9%) and low prevalence of anti-β1R Abs (15.4%) and evoked a marked augmentation of phosphatidylinositol.

Conclusions—Our results demonstrate a strong correlation between anti-β1R Abs and ventricular arrhythmias and anti-M2R Abs and sinus node dysfunction. Anti-β1R Abs increase and anti-M2R Abs inhibit cAMP production. These findings offer new insight into the etiology and pathophysiology of cardiac arrhythmias, with therapeutic implications. (Circulation. 2001;103:1765-1771.)

Key Words: antibodies ■ arrhythmia ■ receptors, adrenergic, beta ■ receptors

Circulating antibodies with agonist-like properties on cardiac autonomic membrane receptors have been reported in heart diseases in which ventricular tachyarrhythmias and bradyarrhythmias are common.1–5 An enhanced sympathetic drive of the heart and catecholamines play a major role in the pathophysiology of ventricular arrhythmias,6,7 whereas an augmented vagal tone and strong vagal stimuli depress sinus node activity and AV nodal propagation.8,9 Thus, it can be hypothesized that the antibodies that stimulate β-adrenergic and M 2 -cholinergic receptors might participate in the pathogenesis of ventricular ectopy and bradyarrhythmias, respectively. Anti-β-adrenergic receptor antibodies were related to ventricular arrhythmias (VAs) in idiopathic dilated cardiomyopathy, whereas anti-M 2 -cholinergic receptor antibodies were associated with chagasic or idiopathic sinus node dysfunction (SND)4 and other arrhythmias found in Chagas’ heart disease.5 Nevertheless, neither the prevalence nor the functional and biochemical effects of these antibodies in different cardiac arrhythmias have been systematically investigated.

The aim of this study was to explore a link between circulating antiautonomic membrane receptor antibodies and the arrhythmias (ventricular ectopy or SND) observed in selected cardiac disorders and to investigate the functional and biochemical effects of these antibodies.

Methods

Patient Population
Forty-one patients and 10 healthy volunteers were studied (Table 1). Group I consisted of 28 patients with VA occurring in a structurally normal heart (14 patients) or caused by chronic Chagas’ heart disease (9 patients) or idiopathic dilated cardiomyopathy (5 patients). Group II comprised 13 patients with SND, either primary (4 patients) or chagasic (9 patients). Group III was composed of 10 healthy controls.

The evaluation included a clinical and cardiological examination, serological tests for Chagas’ infection, an ECG at rest, a B-mode echocardiogram, 201Tl scintigraphy, and 24-hour ECG Holter monitoring. Coronary angiography was performed in the 5 patients with idiopathic dilated cardiomyopathy, and 25 patients (12 with VA and 13 with SND) underwent an electrophysiological study.
TABLE 1. Clinical Characteristics of the Study Patients

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Patients</th>
<th>Age, y</th>
<th>Sex</th>
<th>Type of Arrhythmia</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (VA)</td>
<td>28</td>
<td>46.2 (31–68)</td>
<td>F: 18</td>
<td>Frequent PVCs</td>
<td>PVCs: 14 pts</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M: 10</td>
<td></td>
<td>PVCs: 9 pts</td>
</tr>
<tr>
<td>II (SND)</td>
<td>13</td>
<td>45.4 (22–68)</td>
<td>F: 9</td>
<td>SND</td>
<td>Primary SND: 4 pts</td>
</tr>
<tr>
<td>III (HC)</td>
<td>10</td>
<td>45.5 (30–65)</td>
<td>F: 5</td>
<td>None</td>
<td>Chronic CH: 9 pts</td>
</tr>
</tbody>
</table>

HC indicates healthy controls; PVCs, premature ventricular contractions; and pts, patients.

Chronic Chagas’ heart disease and idiopathic dilated cardiomyopathy were diagnosed according to reported criteria. The arrhythmias were regarded as primary whenever no structural abnormalities of the heart were detected and serological tests for Chagas’ disease were negative.

Patients with >30 ventricular extrasystoles per hour were included (range 866 to 18 615/24 hours). Ventricular ectopy was uniform in 14 patients and multifocal in the other 14. Repetitive ventricular activity was documented in 20 patients. Five patients had experienced 1 or more episodes of sustained ventricular tachycardia, and in 4 of them, the arrhythmia was induced by programmed ventricular stimulation.

SND was diagnosed as reported previously, either when VAs were absent (10 patients) or when a nonsignificant number of ventricular extrasystoles (<1/hour; 3 patients) were recorded.

After clinical evaluation, blood samples were drawn, and sera were obtained and coded. The IgG fractions and antiautonomic receptor antibodies were purified and their functional and biochemical effects evaluated.

The study protocol was approved by the Ramos Mejía Hospital Ethical Board. Each patient was informed about the study characteristics and signed a written consent form.

IgG Fractionation
IgG fractions were separated by ammonium sulfate precipitation and dialyzed against PBS. Less than 10% contamination with IgM was demonstrated by an enzyme immunoassay.

Antibody Immunopurification
Antibodies against M₃-cholinergic and β-adrenergic receptors were affinity purified from the IgG with synthetic peptides corresponding to the second extracellular loop of the receptors (M₃: VRTVEDGE-CYIQFFSNAAVTFGTAI; β: HWusraesdarecynndpkccd-FVTNR) coupled to activated CNBr Sepharose.

Functional Assay
The chronotropic effects of IgG and immunopurified antibodies were assessed on spontaneously beating cultured cardiomyocytes. The baseline beating rate, measured in 10 fields at 37°C, was 120 ± 24 bpm. Measurements were repeated 1 hour after exposure to IgG at a 1:50 dilution and subsequent addition of atropine (10⁻⁷ mol/L), propranolol (10⁻⁵ mol/L), and isoproterenol (10⁻⁷ mol/L). Functional tests were performed in a blinded manner 3 times for reproducibility.

The functional test was considered positive for the presence of anti-β-adrenergic receptor antibodies if addition of IgG induced a statistically significant increase in the beating rate (compared with the control IgG) that was neutralized by propranolol. It was considered positive for the presence of anti-M₃-cholinergic receptor antibodies whenever addition of IgG led to a statistically significant reduction in the beating rate (compared with the control IgG) that was antagonized by atropine.

IgG from a single patient may contain both anti-M₃-cholinergic and anti-β-adrenergic receptor antibodies, whose chronotropic effects may counteract each other. Therefore, a significant increase in the beating rate of cardiomyocytes after exposure to atropine that was in turn antagonized by propranolol, even in the absence of any initial significant change induced by the IgG alone, was interpreted as an indication of the presence of both types of antiautonomic receptor antibodies.

Biochemical Studies
Expression Vectors and Transfection
COS7 cells were transfected with pBC expression vector containing cDNA encoding for the human β₁-adrenergic receptor, containing the gene of the rat β₂-adrenergic receptor. For phosphatidylinositol (PI) turnover, cells were cotransfected with OB vector containing M₃-cholinergic receptor cDNA plus pCDNAI vector having the cDNA encoding for a chimera of murine Gₛₐ and Gₛ₃₅ transduction proteins or with pCEF containing the M₃-cholinergic receptor cDNA as control. The pCMV-β-Gal plasmid was used to evaluate basal receptor expression and transfection efficiency, estimated as 75% by a β-Gal colorimetric method. Plasmids were amplified in Escherichia coli XL1 Blue (Stratagene) and purified by column chromatography (Qiagen Maxiprep kits). One microgram of the expression plasmid was used to transfect COS7 cells, as described previously.

Measurement of cAMP and PI Hydrolysis
The principle of competitive protein binding was used to assess cAMP production in β₁- and β₂-adrenergic transfectants after incubation with 10⁻⁷ mol/L isoproterenol for 10 minutes or with a 1:50 final dilution of the IgG for up to 60 minutes, and finally expressed as pmol/tube. M₃+Gₛₐ cotransfectants and M₃ transfectants (control) incubated 1 hour with 50 μmol/L carbachol or the IgG were used to evaluate PI hydrolysis, as described previously. β-Gal transfectants were used as controls for each experiment.

Statistical Analysis
Changes in the beating rate were assessed by ANOVA and Tukey test. A MANOVA test was used to compare the results between groups (Software Statistica 5.1, 1997). P < 0.05 was chosen as the least significant difference. Changes in cAMP and PI production were evaluated by nonparametric tests (Kruskal-Wallis and Dums) and the Mann-Whitney rank sum test, respectively.

Results
Prevalence of Antiautonomic Membrane Receptor Antibodies in Relation to Cardiac Arrhythmias
Figure 1 depicts the chronotropic effects of the IgG fractions. The beating rate was not modified by the IgG from the healthy controls, whereas it was increased by the IgG from patients with VA and decreased by the IgG from patients with SND (P < 0.001 between groups), irrespective of the origin of the arrhythmias.
Figure 2 shows the prevalence of antiautonomic membrane receptor antibodies in the different study groups and according to the cause of the arrhythmias. No antiautonomic membrane receptor antibodies were detected in the healthy controls. Patients with VA showed a high prevalence of anti-β-adrenergic receptor antibodies (75%; P < 0.001 versus groups II and III) and a relatively low prevalence of anti-M2-cholinergic receptor antibodies (10.7%; P < 0.05 versus group III). The prevalence of anti-β-adrenergic receptor antibodies was similar for patients with primary VA (72%), Chagas’ heart disease (78%), and idiopathic dilated cardiomyopathy (80%), whereas anti-M2-cholinergic receptor antibodies occurred only in the first subgroup (22%). In marked contrast, patients with SND had a high prevalence (76.5%) of anti-M2-cholinergic receptor antibodies (75% for the subgroup with primary SND and 78% for chagasic patients; P < 0.001 versus groups I and III) and a low prevalence (15.4%) of anti-β-adrenergic receptor antibodies, which were present only in chagasic patients (22%; P < 0.10 versus group III). Thus, anti-β-adrenergic receptor antibodies were significantly more prevalent in patients with VA than in those with SND, whereas the opposite occurred regarding anti-M2-cholinergic receptor antibodies.

Table 2 shows the effects of atropine on the electrocardiographic and electrophysiological manifestations of SND as related to the presence of antiautonomic membrane receptor antibodies. Although the number of patients did not allow reliable statistical analysis, the prevalence of anti-M2-cholinergic receptor antibodies did not differ substantially in patients in whom atropine corrected the manifestations of SND totally or in part. The same occurred regarding anti-β-adrenergic receptor antibodies in patients with inducible versus noninducible sustained ventricular tachycardia.

Figure 3 illustrates the correlation of the electrocardiographic findings with the in vitro effects of the IgG from 2 patients, 1 with a “pure” primary SND (A) and the other with chagasic VA (B). In Figure 3A, a pronounced sinus bradycardia that was corrected by atropine (top) and the chronotropic effect of the IgG fraction on cultured cardiomyocytes before and after addition of atropine (bottom) were concordant with the clinical findings. The marked slowing of the beating rate induced by the IgG was neutralized by atropine, indicating the existence of anti-M2-cholinergic receptor antibodies. In B, a selected strip from a 24-hour ECG Holter monitoring shows the characteristics of the ventricular ectopy (top). A 95% suppression of ventricular extrasystoles was achieved with nadolol at a daily dose of 80 mg (from 3765 at baseline to 188 after 7 weeks of treatment), suggesting that the arrhythmia was adrenergically driven. Furthermore, the chronotropic action of the IgG from this patient was abolished by propranolol (bottom), denoting the presence of anti-β-adrenergic receptor antibodies.

Analysis of the effects of the IgG on an individual basis revealed a broad spectrum of quantitative and qualitative chronotropic changes (Figure 4). Furthermore, the chronotropic effects of the IgG fractions from 9 patients having anti-β-adrenergic receptor antibodies and from 2 with anti-M2-cholinergic receptor antibodies were not totally abolished by the corresponding autonomic receptor antagonist. This was not due to low concentrations of atropine or propranolol in the assay, because concentrations >1 μmol/L caused a toxic effect (absence of chronotropic activity or slow and irregular rhythm). Therefore, persistence of a mild or moderate, but still significant, chronotropic effect of an IgG after addition of atropine or propranolol would suggest the presence of antibodies acting on membrane receptors other than anti-M2-cholinergic and anti-β-adrenergic receptor antibodies in 3 study groups and subgroups. Bars represent percentage of IgG containing anti-β-adrenergic (open bars) or anti-M2-cholinergic (solid bars) receptor antibodies. PVA indicates primary VA; PSND, primary SND; Ch, Chagas’ heart disease; IDC, idiopathic dilated cardiomyopathy; and HC, healthy controls.
the M₂-cholinergic or the β-adrenergic receptor and/or on ionic channels that influence cardiomyocyte automatism.

The results obtained with the IgG before and after addition of atropine and propranolol indicate indirectly the presence of antiautonomic receptor antibodies. Functional testing of the immunopurified antibodies in 10 selected cases was perfectly concordant with that performed with the IgG (Table 3).

**Changes in Second Messenger Production**

In Figure 5, the biochemical effects of the IgG containing β₁- and/or β₂-adrenergic receptor antibodies or anti-M₂-cholinergic receptor antibodies were compared with those of the IgG from healthy controls. The latter did not modify cAMP or PI production. The IgG from patients with VAs increased cAMP in COS7 cells transfected with either β₁-adrenergic receptors (2.8±1.1-fold versus 0.25±0.36-fold in patients with SND; *P*<0.001) or β₂-adrenergic receptors (7.4±5.4-fold versus 0.55±0.26-fold in patients with SND; *P*<0.001) without affecting PI concentration. In contrast, the IgG from patients with SND only increased PI production (5.8±4.5-fold versus 0.13±0.12-fold in patients with VAs; *P*<0.01). Thus, anti-β₁- and anti-β₂-adrenergic receptor antibodies stimulate the corresponding membrane receptor that interacts with the Gₛ protein to induce an increment of cAMP. Anti-M₂-cholinergic receptor antibodies stimulate the corresponding receptor that interacts with the Gᵢ protein, inhibiting cAMP production. These actions explain the chronotropic action of the antibodies on cardiomyocytes. The long-lasting

### TABLE 2. Effects of Atropine on Electrophysiological Manifestations of SND as Related to Antiautonomic Receptor Antibodies

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Diagnosis</th>
<th>Heart Rate, bpm</th>
<th>SNRT</th>
<th>Antibodies</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>Baseline</td>
<td>Atropine</td>
<td>Baseline</td>
</tr>
<tr>
<td>192</td>
<td>PSND</td>
<td>44</td>
<td>100</td>
<td>855</td>
</tr>
<tr>
<td>240</td>
<td>PSNP</td>
<td>50</td>
<td>110</td>
<td>580</td>
</tr>
<tr>
<td>176</td>
<td>PSND</td>
<td>45</td>
<td>95</td>
<td>325 (SP)</td>
</tr>
<tr>
<td>106</td>
<td>PSND</td>
<td>42</td>
<td>102</td>
<td>470 (SP)</td>
</tr>
<tr>
<td>211</td>
<td>ChSND</td>
<td>42</td>
<td>72</td>
<td>480 (SP)</td>
</tr>
<tr>
<td>94</td>
<td>ChSND</td>
<td>52</td>
<td>104</td>
<td>690</td>
</tr>
<tr>
<td>2</td>
<td>ChSND</td>
<td>45</td>
<td>95</td>
<td>750</td>
</tr>
<tr>
<td>15</td>
<td>ChSND</td>
<td>36</td>
<td>75</td>
<td>1150</td>
</tr>
<tr>
<td>29</td>
<td>ChSND</td>
<td>48</td>
<td>98</td>
<td>610</td>
</tr>
<tr>
<td>62</td>
<td>ChSND</td>
<td>40</td>
<td>72</td>
<td>785</td>
</tr>
<tr>
<td>83</td>
<td>ChSND</td>
<td>42</td>
<td>98</td>
<td>690</td>
</tr>
<tr>
<td>199</td>
<td>ChSND</td>
<td>38</td>
<td>80</td>
<td>790</td>
</tr>
<tr>
<td>217</td>
<td>ChSND</td>
<td>45</td>
<td>85</td>
<td>AER</td>
</tr>
</tbody>
</table>

**SNRT** indicates corrected sinus node recovery time; **Anti-M₂**, anti-M₂-cholinergic receptor antibodies; **Anti-β**, anti-β-adrenergic receptor antibodies; **PSND**, primary SND; **SP**, secondary pauses; **ChSND**, chagasic SND; **AER**, atrial ectopic rhythm; and **JR**, junctional rhythm.

**Figure 3.** Correlation between ECG findings (top) and chronotropic effects of IgG (bottom) in patient with “pure” primary SND (A) and another with chronic Chagas’ heart disease, VAs, and normal sinus node function (B). ECG leads I, II, III, and V₁ were simultaneously recorded with high right atrium (HRA) and His bundle (HBE) electrograms. C₁, C₂, and C₂ represent Holter ECG leads; HR indicates heart rate; atrop, atropine; and prop, propranolol. Bars represent beating rate of cardiomyocytes.
increment of cAMP induced by the anti-β-adrenergic receptor antibodies contrasts with the ephemeral action of isoproterenol (Figure 6).

**Discussion**

Our results demonstrate a strong correlation between circulating anti-β-adrenergic receptor antibodies and VA in the setting of a structurally normal heart, idiopathic dilated cardiomyopathy, and Chagas’ heart disease, as well as between circulating anti-M2-cholinergic receptor antibodies and primary or chagasic SND. Anti-β-adrenergic receptor antibodies are rare in patients without VA, whereas anti-M2-cholinergic receptor antibodies are rare in patients with normal sinus node function. Furthermore, anti-M2-cholinergic receptor antibodies are uncommon in patients with “pure” VA, whereas anti-β-adrenergic receptor antibodies are rarely present in patients with “pure” SND.

IgG from a number of patients with either VA or SND contains both anti-β-adrenergic receptor and anti-M2-cholinergic receptor antibodies, whose functional effects on cardiac cells may neutralize each other. In 5 patients (not included in this study) who had both VA and SND, the prevalence of anti-M2-cholinergic and anti-β-adrenergic receptor antibodies was 80% and 40%, respectively. Under such circumstances, the antibodies should not show any potential arrhythmogenic action, thus invalidating the relationship between arrhythmias and antibodies. However, it may be postulated that the net functional effect of the IgG on cardiac tissues is dependent not only on the relative concentration of each type of antiautonomic membrane receptor antibody but also on the relative density of β-adrenergic and M2-cholinergic receptors in the different heart tissues. The functional actions of anti-M2-cholinergic receptor antibodies should prevail in the sinus and AV nodes, rich in cholinergic innervation and receptors. In fact, the inhibitory action of the vagal stimulation on the sinus node prevails over the sympathetic stimulation when both systems are activated simultaneously.18,19 Conversely, the functional effects of β-adrenergic receptor antibodies should predominate in the ventricle, where β-adrenergic innervation and receptors are prevalent.20 Accordingly, the presence of both types of antiautonomic membrane receptor antibodies demonstrated in patients with Chagas’ heart disease13 may explain the coex-

**TABLE 3. Antiautonomic Receptor Antibodies Detected in the IgG and Immunopurified Antibodies**

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>Patient Code</th>
<th>Anti-M2</th>
<th>Anti-β</th>
<th>Immunopurified Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary VA</td>
<td>71</td>
<td>+</td>
<td>+</td>
<td>β1, M2 in flowthrough</td>
</tr>
<tr>
<td>Primary VA</td>
<td>157</td>
<td>-</td>
<td>+</td>
<td>β1</td>
</tr>
<tr>
<td>Primary VA</td>
<td>86</td>
<td>-</td>
<td>+</td>
<td>β1</td>
</tr>
<tr>
<td>Chagasic VA</td>
<td>183</td>
<td>-</td>
<td>+</td>
<td>β1</td>
</tr>
<tr>
<td>Chagasic VA</td>
<td>107</td>
<td>-</td>
<td>+</td>
<td>β1</td>
</tr>
<tr>
<td>IDC VA</td>
<td>9</td>
<td>-</td>
<td>+</td>
<td>β1</td>
</tr>
<tr>
<td>Chagasic SND</td>
<td>29</td>
<td>+</td>
<td>+</td>
<td>M2, β1 in flowthrough</td>
</tr>
<tr>
<td>Chagasic SND</td>
<td>199</td>
<td>+</td>
<td>-</td>
<td>M2</td>
</tr>
<tr>
<td>Chagasic SND</td>
<td>2</td>
<td>+</td>
<td>-</td>
<td>M2</td>
</tr>
<tr>
<td>Primary SND</td>
<td>192</td>
<td>+</td>
<td>-</td>
<td>M2</td>
</tr>
</tbody>
</table>

IDC indicates idiopathic dilated cardiomyopathy; Anti-M2/Anti-β, anti-M2-cholinergic or anti-β-adrenergic receptor antibodies present in the IgG (+).

**Figure 4.** Chronotropic effects of IgG from entire studied population. Bars represent change in beating rate caused by IgG from each individual patient and by IgG from 10 healthy controls (HC, mean value). ChHD indicates Chagas’ heart disease; IDC, idiopathic dilated cardiomyopathy.

**Figure 5.** cAMP and PI production in β1- and β2-adrenergic and M2-cholinergic receptor COS7 transfectants when incubated with IgG from patients with VAs (containing anti-β-adrenergic receptor antibodies) or SND (having anti-M2-cholinergic receptor antibodies) and from healthy controls (without antibodies). IgG from patients with VAs increased cAMP production in β1- or β2-adrenergic transfectants. IgG from patients with SND increased PI turnover in COS7 cells cotransfected with M2-cholinergic receptor and G_{i/q} genes (M2+G_{i/q}). HC indicates healthy controls.
biochemical, arrhythmogenic, and cardiomyopathic effects of antiautonomic membrane receptor antibodies

The functional and biochemical tests indicate that antiautonomic membrane receptor antibodies stimulate the corresponding cardiac receptors. IgG containing anti-β-adrenergic receptor antibodies induces a long-lasting increment of cAMP in β1- and β2-transfectants, as demonstrated in CHW cells for anti-β1-adrenergic receptor antibodies from patients with idiopathic dilated cardiomyopathy.22 In contrast, IgG containing anti-M2-cholinergic receptor antibodies decreased cAMP production in M2 transfectants, as shown in membranes of rat heart ventricles.23 The catecholamine-like and acetylcholine-like actions of the antibodies might play a major role in the pathophysiology of VA and SND, respectively. Nevertheless, no direct or conclusive proofs of the potential arrhythmogenic properties of antiautonomic membrane receptor antibodies are available. A recent report by Farias de Oliveira et al5 showed that the IgG from chagasic patients causes bradycardia and AV block in a Langendorff preparation of the rabbit heart. Interestingly, in our patients with SND and anti-M2-cholinergic receptor antibodies, the electrophysiological abnormalities were totally or partially corrected by atropine. Furthermore, in 4 of our patients with VA and anti-β-adrenergic receptor antibodies, nadolol (80 to 160 mg/d orally) suppressed ventricular ectopy by >90% (baseline 2515 to 7085 per 24 hours, nadolol 51 to 564 per 24 hours).

The antiautonomic membrane receptor antibodies might play a role in the pathogenesis of cardiac arrhythmias not only via their functional effects on the receptors and changes in the electrophysiological properties of cardiac tissues, but also by causing structural damage to the myocardial cells. Matsui et al24 reported that chronic immunization of rabbits with β1- or M2-peptides induces a cardiomyopathy with ventricular dilation and the histological pattern of chronic myocarditis and that 2 of 8 rabbits immunized with the β1-peptide died suddenly. The cardiomyopathic effects of immunization with the peptides may be prevented by simultaneous treatment with specific antagonists. In addition, immunoadsorption dramatically improves myocardial performance and left ventricular dimensions in patients with heart failure due to idiopathic dilated cardiomyopathy and circulating anti-β1-adrenergic receptor antibodies.25,26

The nature of the immunologic disorder causing antiautonomic membrane receptor antibodies

Experimental evidence indicates that β-adrenergic and M2-cholinergic receptors may be immunogenic.24,27 However, the factors that trigger the immunoregulatory abnormality involving the autonomic membrane receptors in cardiac diseases are unknown. The observations of Kaplan et al28 shed light on the nature of the antibodies that recognize and stimulate the autonomic membrane receptors of cardiac cells in patients with Chagas’ heart disease. They demonstrated that the antibodies directed against a ribosomal P protein of Trypanosoma cruzi cross-react with and stimulate the β1-adrenergic receptors. This was attributed to a stretch of acidic residues present in the R13 peptide of the parasite (EEEDDD), which is homologous to an epitope of the second extracellular loop of the β1-adrenergic receptor (AESDE). Therefore, antiautonomic receptor antibodies may actually be directed against antigens of an infectious agent and may not be the result of an autoimmune reaction involving the β1-adrenergic and/or M2-cholinergic receptors.

Implications

In accordance with our results, it is possible to speculate about a potential pathogenic link between antiautonomic membrane receptor antibodies and cardiac arrhythmias found in diverse heart diseases. This approach may open new research avenues and contribute to the knowledge of the etiology and pathophysiology of cardiac arrhythmias, which may have therapeutic consequences. Demonstration of the arrhythmogenic activity of these antibodies remains an issue that merits further clinical and experimental investigations.

Figure 6. Effects of IgG from 3 patients (IgG1 to 3) with VAs (containing anti-β-adrenergic receptor antibodies) and healthy control (HC) on cAMP production in β1- and β2-adrenergic transfectants, compared with isoproterenol. Although less prominent, the stimulating action of antibodies lasted longer than that of isoproterenol.
Acknowledgments
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References
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