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High Prevalence of Antibodies Against Beta$_1$- and Beta$_2$-Adrenoceptors in Patients With Primary Electrical Cardiac Abnormalities

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Objectives. **This study sought to determine the prevalence of autoantibodies directed against the beta-adrenoceptors in patients with primary electrical cardiac abnormalities, including atrial arrhythmias, ventricular arrhythmias and conduction disturbances, in the absence of any other cardiac abnormality.**

Background. **Using synthetic peptides corresponding to the predicted sequences for the second extracellular loop of the human beta$_1$- and beta$_2$-adrenoceptors as antigenic targets, autoantibodies directed against the beta-adrenoceptors were recently shown to occur in patients with idiopathic dilated cardiomyopathy and Chagas' heart disease.**

Methods. **Eighty-six patients (57 with primary electrical abnormalities, 29 with idiopathic dilated cardiomyopathy) and 101 healthy and cardiopathic control subjects were studied. Antibodies against the beta$_1$- and beta$_2$-peptides were detected with an enzyme immunoassay performed in blinded manner. In nine selected (seropositive) cases, the immunoglobulin G (IgG) fraction was tested for functional effects on the rate of beating of cultured neonatal rat cardiomyocytes.**

Results. **Antibodies recognizing the beta$_1$- and beta$_2$-peptides were found in 11 (52.3%) of 21 patients with ventricular arrhythmias ($p < 0.01$), 5 (35.7%) of 14 patients with conduction disturbances ($p < 0.05$), 3 (13.6%) of 22 patients with atrial arrhythmias ($p > 0.05$) and 11 (37.9%) of 29 patients with dilated cardiomyopathy ($p < 0.05$) compared with 15 (14.8%) of 101 control subjects. A rapid increase in the rate of beating of the cultured cardiomyocytes was induced by IgG from a selected group of patients, suggesting an agonist-like interaction with a functional epitope. This response was mediated by stimulation of both the beta$_1$- and beta$_2$-adrenoceptors in the patients with primary ventricular arrhythmias but only the beta$_1$-adrenoceptors in the patients with idiopathic dilated cardiomyopathy.**

Conclusions. **Primary ventricular arrhythmias and conduction disturbances, like idiopathic cardiomyopathy, show a high prevalence of antibodies interacting with functional epitopes of the beta-adrenoceptors, suggesting a common or similar abnormal immunoregulatory process.**

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The past decade has seen considerable progress in the delineation of the structure of beta-adrenoceptors. The primary sequences of the beta$_1$- and beta$_2$-adrenoceptors were derived from the corresponding DNA sequences (1–3), and the second extracellular loop was postulated to contain the T and B cell epitopes necessary for induction of an immune response (4). Two synthetic peptides corresponding to the predicted sequences for the second extracellular loop of the human beta$_1$- and beta$_2$-adrenoceptors were used as antigenic targets to screen for receptor-specific antibodies in European patients with idiopathic dilated cardiomyopathy (5), a condition in which an autoimmune mechanism has been considered to play a major role (6–8). Using an enzyme immunoassay, sera from patients with idiopathic dilated cardiomyopathy were shown to monospecifically recognize the beta$_1$-peptide (5). The affinity-purified antibodies had an inhibitory effect on radioligand binding to the beta$_1$-adrenoceptor of C6 rat glioma cells, recognized the receptor protein by immunoblot and bound in situ to human myocardial tissue. Antibodies recognizing both the beta$_1$- and beta$_2$-peptides were later shown to be highly prevalent in patients with Chagas' heart disease (9,10), a specific form of dilated cardiomyopathy in which an autoimmune mechanism has also been implicated (11,12).

In view of the demonstration of anti–beta-adrenoceptor antibodies in these two etiologically different forms of cardiomyopathy, we investigated the presence of these antibodies in patients with cardiac arrhythmias and conduction disturbances unrelated to cardiomyopathy or any other manifest evidence of cardiac involvement. Such conditions are well known to the clinical cardiologist, who often has difficulty defining the etiology and pathogenesis of these complications,

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Table 1. Seropositive Results in 57 Patients With Primary Electrical Abnormalities

	Electrical Abnormality	No. of Pts	No. of Pts With Positive Sera
Group 1a (atrial arrhythmias [22 pts])	Paroxysmal atrial fibrillation and/or flutter	7	0
	Frequent atrial premature beats (≥1,000/24 h on Holter ECG)	12	3
	Sinus node dysfunction	3	0
Group 1b (ventricular arrhythmias [21 pts])	Frequent, uniform, nonrepetitive PVCs (≥30/h on Holter ECG)	6	3
	Frequent, uniform, repetitive PVCs	4	1
	Frequent, multiform, nonrepetitive PVCs	2	0
	Frequent, multiform, repetitive PVCs	8	6
	VT/VF	1	0
Group 1c (conduction disturbances [14 pts])	Intraventricular block*	10	3
	AV block†	4	2

*Includes five patients (Pts) with left bundle branch block, four with left anterior hemiblock (one with coexisting rate-dependent left bundle branch block) and one with right bundle branch block plus left anterior hemiblock. †Includes three patients with complete atrioventricular (AV) block and one with second-degree AV block. ECG = electrocardiogram; PVCs = premature ventricular contractions; VF = ventricular fibrillation; VT = ventricular tachycardia.

which include frequent atrial and ventricular arrhythmias, sinus node dysfunction and various forms of intraventricular and atrioventricular (AV) block. These conditions may be lumped together as "primary electrical cardiac abnormalities." Our major objective was to study the prevalence of anti–beta-adrenoceptor antibodies in patients with these electrical abnormalities compared with healthy and cardiopathic control subjects and with a new series of patients with idiopathic dilated cardiomyopathy.

Methods

One hundred eighty-seven patients, all recruited at Ramos Mejía Hospital in Buenos Aires, were selected and allocated to one of three groups. Group 1 included 57 patients with primary electrical abnormalities (31 men, 26 women; mean age 41.7 years, range 15 to 73). Criteria for inclusion were the presence of atrial arrhythmias (group 1a), ventricular arrhythmias (group 1b) or conduction disturbances (group 1c) (Table 1). The absence of any other cardiac abnormality (or any other acute or chronic illness) was established in all patients on the basis of 1) careful history and clinical examination; 2) normal routine laboratory tests (including serology for Chagas' disease); 3) absence of any electrocardiographic changes other than the arrhythmias and conduction disturbances; 4) normal findings on the echocardiogram; and 5) a normal response to exercise testing. Group 2 included 101 control subjects of whom 68 were healthy volunteers and mostly blood donors (group 2a [57 men, 11 women; mean age 32.9 years, range 20 to 43]), and 33 had diverse cardiac conditions other than idiopathic dilated cardiomyopathy or Chagas' disease (group 2b [25 men, 8 women; mean age 58.2 years, range 28 to 79]) (Table 2). Group 3 included 29 patients with idiopathic dilated cardiomyopathy (18 men, 11 women; mean age 45.1 years, range 14 to 75) and was included in the study for purposes of comparison between primary electrical abnormalities and idiopathic dilated cardiomyopathy (as for control subjects) in patients from the same geographic area. The diagnosis of idiopathic dilated cardiomyopathy was based on previously reported criteria (13).

Immunoserologic test. After all clinical studies were completed, blood samples were obtained, and the sera were diluted in 50% (vol/vol) glycerol and stored at −20°C until use. When a satisfactory number of sera were collected (30 to 90 days after blood extraction), the sera were processed for detection of antibodies directed against the synthetic $beta_1$- and $beta_2$-peptides by using previously described enzyme immunoassay (5). Briefly, peptides were synthesized using an automatic peptide synthesizer, desalted and stored at −20°C until use. The peptides correspond to the hypothesized second extracellular loop of the human beta-receptors. The $beta_1$-peptide corresponds to the sequence of amino acids 183 to 208 of the $beta_1$-adrenoceptors (H-W-W-R-A-E-S-D-E-A-R-R-C-Y-N-D-P-K-C-C-D-F-V-T-N-R) and the $beta_2$-peptide to the sequence 172 to 197 of the $beta_2$-adrenoceptors (H-W-Y-R-A-

Table 2. Seropositive Results and Clinical Diagnosis in 33 Cardiopathic Control Subjects

Clinical Diagnosis	No. of Pts	No. of Pts With Positive Sera
Hypertrophic cardiomyopathy	15	2
Ischemic heart disease	7	0
Valvular heart disease	5	0
Hypertensive heart disease	2	0
Alcoholic cardiomyopathy	1	0
Cardiac sarcoidosis	1	0
Kawasaki disease	1	0
Familial periodic paralysis with ventricular arrhythmias	1	0

Pts = patients.

T-H-Q-E-A-I-N-C-Y-A-N-E-T-C-C-D-F-F-T-N-Q). Fifty microliters of 0.1 mol/liter sodium carbonate solution supplemented with 1% (vol/vol) beta-mercaptoethanol containing 50 μg/ml of peptide was absorbed for 1 h at room temperature on microtiter plates. The wells were then saturated with phosphate-buffered saline solution [16 mmol/liter phosphate, 150 mmol/liter sodium chloride, pH 7.4]) supplemented with 3% (vol/vol) skim milk, 0.1% (vol/vol) Tween-20 and 0.01% (vol/vol) thimerosal (PMT). Fifty microliters of dilutions of sera from 1:20 to 1:1600 were allowed to react with the peptides overnight at 4°C. After washing the wells three times with phosphate-buffered saline solution, 0.05 ml of an affinity-purified biotinylated rabbit anti-human immunoglobulin G (IgG) antibody solution diluted 1:1, 000 in PMT was allowed to react for 1 h at room temperature. After three additional washings, the bound biotinylated antibody was detected by incubation of the plates for 1 h at room temperature with 0.05 ml/well of a 1-μg/ml solution of streptavidin-peroxidase in PMT followed by three washings in phosphate-buffered saline solution and addition of the chromogenic substrate hydrogen peroxide (2.5 mmol/liter) 2,2′-azino-di-(ethylbenzthiazoline) sulfonic acid (2 mmol/liter). Thereafter, 30-min optical densities were read at 405 nm in a Micro Elisa Reader (Molecular Devices).

All determinations were performed in the same laboratory (Wallenberg Laboratory, Sahlgren's Hospital, Göteborg, Sweden) in four separate batches (during a period of 18 months) under blinded conditions except for the healthy control subjects. Each batch of sera included 10% to 50% from the healthy control subjects. Sera were considered positive whenever optical density values exceeded the mean value + 2 SD of optical densities obtained for the healthy control subjects, and after exclusion of those whose optical density values exceeded this limit, which were considered as positive healthy control sera.

Functional test. In nine selected patients (four with primary electrical abnormalities, four with idiopathic dilated cardiomyopathy, one healthy control subject) whose sera were shown to be positive, the IgG fraction was isolated by ammonium sulfate precipitation and tested for functional (chronotropic) effects on the rate of beating of cultured neonatal rat cardiomyocytes, as previously described (14). Briefly, single cells were dissociated from the minced ventricles with a 0.2% solution of trypsin and were cultured at 37°C for 4 days as monolayers (90,000 cells/cm^2). On the day of the experiment, the medium was replaced, and the cells were incubated for 2 h as monolayers on a slowly moving rocker apparatus. During this stage, cells were sensitized for $beta_2$-adrenoceptor response by addition of 10 mmol/liter of 15-hydroxyeicositetraenic acid (15). The flasks were then transferred to the heated stage of an inverted Zeiss microscope on which 10 small circular fields of the cell layer were inspected at 37°C through the perforations of a metal template. The number of beats of a selected isolated myocardial cell or a cluster of synchronous contracting cells in each of the 10 fields was visually counted for 15 s each time. This procedure was repeated twice in different cultures to yield results representing a total of 10 cells or cell clusters, before and 60 min after preincubation with the IgG at dilutions of 1:10 to 1:100. The counting was repeated after the subsequent and successive addition (without washing) of first the selective $beta_1$-adrenergic blocking agent bisoprolol (1 μmol/liter), and then the selective $beta_2$-blocker ICI 118,551 (30 nmol/liter). All functional tests were performed in a blinded manner.

Statistical analysis. Analysis of variance (a Statview SE+ Graphics TM version 1.03 software package on a Macintosh personal computer) was used to define the statistical significance of differences in the prevalence of anti–$beta_1$ and anti–$beta_2$-adrenoceptor antibodies in the different groups of patients. Significance was assessed at $p < 0.05$. The increase in beating frequency of cultured rat cardiomyocytes compared with the corresponding control period before addition of IgG was analyzed using a paired Student t test. Results are expressed as mean value ± SEM. A probability level of $p < 0.05$ was chosen as the least significant difference.

Results

The prevalence of antibodies directed against the beta-adrenoceptors differed in the three groups of patients with primary electrical cardiac abnormalities. Thus, antibodies recognizing the $beta_1$- or $beta_2$-peptides, or both, were found in 11 (52.3%) of 21 ($p < 0.01$) patients with ventricular arrhythmias, in 5 (35.7%) of 14 ($p < 0.05$) patients with conduction disturbances and in 3 (13.6%) of 22 ($p > 0.05$) patients with atrial arrhythmias compared with 15 (14.8%) of 101 control patients. In patients with idiopathic dilated cardiomyopathy, the prevalence was 11 (37.9%) of 29 ($p < 0.05$). Other differences became apparent when the prevalence of anti–$beta_1$- and anti–$beta_2$-adrenoceptor antibodies was analyzed independently and against the healthy and cardiopathic control subjects (Figure 1). No statistical differences were found in the prevalence of either anti–$beta_1$- or anti–$beta_2$-adrenoceptor antibodies between healthy and cardiopathic control subjects. Antibodies recognizing the $beta_1$-peptide with a difference that was significant against both the healthy and cardiopathic control subjects occurred in patients with primary ventricular arrhythmias as well as in those with idiopathic dilated cardiomyopathy (Fig. 1). In contrast, antibodies recognizing the $beta_2$-peptide occurred only in patients with primary ventricular arrhythmias, with a difference that was significant versus the cardiopathic but not the healthy control subjects. Although it failed to reach statistical significance, a positive trend toward recognition of the $beta_1$-peptide occurred in the patients with conduction disturbances. In contrast to all other groups, the sera of patients with atrial arrhythmias failed to show any recognition of the presence of either anti–$beta_1$- or anti–$beta_2$-adrenoceptor antibodies.

Because ventricular arrhythmias and conduction disturbances occur commonly in patients with idiopathic dilated cardiomyopathy (13), we compared the prevalence of the antibodies in patients with (23 of our 29 patients) and without these abnormalities (the remaining 6 patients). As shown in

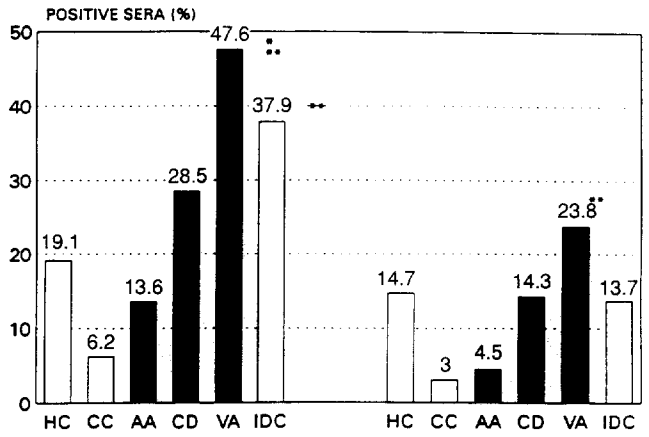
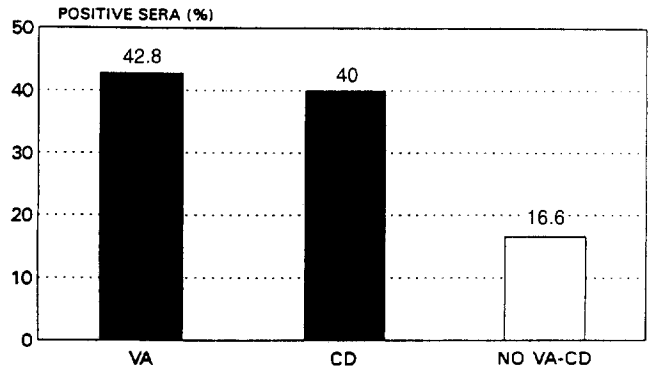


Figure 1. Percent of sera recognizing the anti-beta$_1$- **(left)** and anti-beta$_2$-peptides **(right)** in patients with primary atrial arrhythmias (AA), conduction disturbances (CD), ventricular arrhythmias (VA) and idiopathic dilated cardiomyopathy (IDC) compared with healthy (HC) and cardiopathic control subjects (CC). *$p < 0.05$ versus healthy control subjects. **$p < 0.05$ versus cardiopathic control subjects. See text for further discussion.

Figure 2, the high prevalence of anti–beta-adrenoceptor antibodies occurred only in patients with ventricular arrhythmias and conduction disturbances.

Functional effects of anti–beta-adrenoceptor antibodies. The basal beating rate of cultured rat cardiomyocytes was 140 ± 20 beats/min. As shown in Figure 3, the IgG from two patients with primary ventricular arrhythmias whose sera recognized both the beta$_1$- and beta$_2$-peptides, caused a positive chronotropic effect that was partially neutralized by the selective beta$_1$-blocker bisoprolol and completely abolished by the selective beta$_2$-blocker ICI 118,551. It is clear that the chronotropic effect was mediated by stimulation of both the beta$_1$- and beta$_2$-adrenoceptors. In contrast, the IgG from two patients with idiopathic dilated cardiomyopathy whose sera recognized only the beta$_1$-peptide induced a chronotropic response that was completely abolished by bisoprolol, suggesting that the effect was entirely mediated by stimulation of the beta$_1$-adrenoceptors. An identical response occurred in two other patients with idiopathic dilated cardiomyopathy (not shown) and had previously been reported (17) to result from the use of affinity-purified anti–beta$_1$-adrenoceptor antibodies. In the single patient with primary intraventricular block, whose serum only recognized the beta$_1$-peptide, the responses to IgG and bisoprolol were similar to that in patients with idiopathic dilated cardiomyopathy. In one of the three patients with primary ventricular arrhythmias and in one healthy control subject whose sera recognized only the beta$_1$-peptide, IgG failed to cause any significant chronotropic effect. A similar lack of functional response was documented in four other serologically positive healthy control subjects tested in our previous studies (17), suggesting the existence of "naturally occurring antibodies" that may recognize the synthetic peptides but not the specific functional epitopes.

Figure 2. Percent of sera recognizing the beta$_1$-peptide (POSITIVE SERA) in 29 patients with idiopathic dilated cardiomyopathy, according to the presence or absence of ventricular arrhythmias (VA) and conduction disturbances (CD). One patient had both ventricular arrhythmias and conduction disturbances.



Discussion

Our study confirms the finding that patients with idiopathic dilated cardiomyopathy exhibit a high prevalence of anti-beta-adrenoceptor antibodies and demonstrates that a similarly high or even higher prevalence of such antibodies also occurs in patients with primary ventricular arrhythmias or conduction disturbances, or both. We also showed that IgG from the same patients results in a similar positive chronotropic response (in in vitro experiments), mediated by stimulation of the beta$_1$-adrenoceptors in patients with dilated cardiomyopathy and by stimulation of both the beta$_1$- and beta$_2$-adrenoceptors in patients with primary ventricular arrhythmias. In view of these similarities, we were initially inclined to believe that primary ventricular arrhythmias or conduction disturbances, or both, may represent early or parallel manifestations of idiopathic dilated cardiomyopathy preceding the advanced stage of cardiac failure or abortive forms of the same disease. However, some unexpected findings raised doubts regarding the full validity of this hypothesis. Thus, the high prevalence of the antibodies in our patients with idiopathic dilated cardiomyopathy occurred only in those patients with ventricular arrhythmias or conduction disturbances (Fig. 2). Similarly, when a ligand-binding inhibition assay was used to screen for anti–beta-adrenoceptor antibodies in patients with idiopathic dilated cardiomyopathy (18), 6 (30%) of 20 were seropositive when the dilated cardiomyopathy was associated with complex ventricular arrhythmias, whereas only 1 (5%) of 20 was seropositive when the arrhythmias were absent. These observations seem to suggest that anti–beta-adrenoceptor antibodies correlate better or more directly with ventricular arrhythmias and conduction disturbances than with dilated cardiomyopathy itself.

Potential arrhythmogenicity and cardiotoxicity of antibodies. It has been shown (14,17) that a low concentration (0.02 to 0.41 nmol/liter) of affinity-purified anti–beta$_1$-adrenoceptor antibodies from patients with idiopathic dilated cardiomiopathy or immunized rabbits may cause a strong chronotropic

stimulation (up to 80% of the maximal effect of isoproterenol) in cultured rat cardiomyocytes. Unlike the effect of isoproterenol, the effect of the antibodies persists unchanged for many hours (17). This lack of short-term desensitization may be expected to sustain and reinforce the agonist-like effects of the antibodies. If similar effects occurred in vivo, the antibodies might exert arrhythmogenic as well as cardiotoxic effects like those shown to be caused by catecholamines, although this has yet to be proved in appropriate experimental models.

Reappraisal of primary ventricular arrhythmias and conduction disturbances. In view of their association with anti–beta-adrenoceptor antibodies, the significance of these abnormalities needs to be reevaluated. In several of our patients with advanced idiopathic dilated cardiomyopathy, left bundle branch block or ventricular arrhythmias, or both, had been documented 10 to 20 years earlier, at a time when cardiac size was still normal, suggesting that the electrical abnormalities were indeed the earliest manifestations of the immunopathologic process conducive to the development of overt dilated cardiomyopathy. However, in some patients with primary ventricular arrhythmias or intraventricular block associated with the antibodies, a retrospective follow-up of up to 20 years failed to reveal any deterioration. Prospective follow-up studies will be needed to determine whether such abnormalities in the presence of anti–beta-adrenoceptor antibodies may serve as diagnostic or prognostic markers for idiopathic dilated cardiomyopathy.

Study limitations. *Anti–beta-adrenoceptor antibodies in normal subjects.* The main difficulty in the present study was the relatively high prevalence of antibodies recognizing the $beta_1$- and $beta_2$-peptides in the control subjects. It is also true that the specificity and sensitivity of the enzyme immunoassay utilized in our study have not yet been strictly defined. However, these limitations do not detract from the practical usefulness of this procedure for the screening of anti–beta-adrenoceptors antibodies. Thus, this simple assay has served to single out idiopathic dilated cardiomyopathy, primary ventricular arrhythmias and Chagas' heart disease as the only cardiac conditions associated with a high prevalence of these antibodies (9). The validity of our results is also supported by the following findings:

1. The prevalence of the antibodies in our patients with dilated cardiomyopathy was similar to that previously demonstrated with the same method (5) or with a ligand-binding inhibition assay (16,18).

2. The results were also essentially similar in each of the four batches of sera from all our patients, in regard to both the test and control cases.

3. Correct identification of both the anti–$beta_1$- and anti–$beta_2$-adrenoceptor antibodies was demonstrated (Figure 3) in the seropositive tests counterchecked with a functional test. However, the functional test is too complex and labor intensive to be useful in a large number of patients, as in our study.

4. Conversely, the seropositive results in the control subjects seem to imply the existence of natural antibodies deprived of any functional effect. In fact, these "false" seropositive results tend to become negative when enzyme immunoassay is performed utilizing IgG instead of the full serum (unpublished observations).

Conclusions and clinical implications. Primary ventricular arrhythmias and conduction disturbances, like idiopathic dilated cardiomyopathy, are associated with a high prevalence of antibodies interacting with functional epitopes mapped to the second extracellular loop of the beta-adrenoceptors. Therefore, these cardiac abnormalities seem to be interrelated through a similar abnormal immunoregulatory process involving the beta-adrenoceptors. The antibodies exert agonist- or catecholamine-like effects that might facilitate the occurrence of ventricular arrhythmias and play a role in the development of dilated cardiomyopathy. These observations open new insights into the pathogenesis, diagnosis, prognosis and treat-

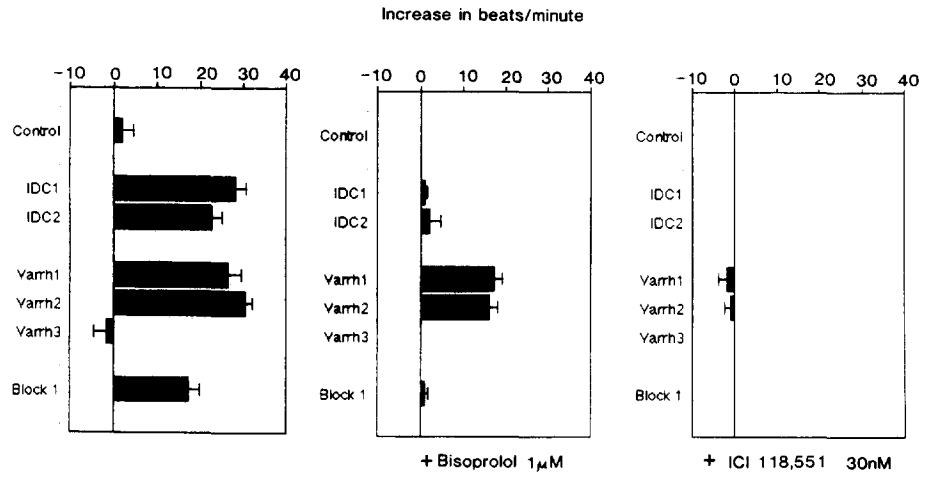


Figure 3. Positive chronotropic effect (increase in beats/minute) on cultured neonatal rat cardiomyocytes **(left panel)** induced by immunoglobulin G obtained from a selected group of seropositive patients. Sera from a single healthy control subject, two patients with idiopathic dilated cardiomyopathy (IDC1 and IDC2), the third patient with primary ventricular arrhythmias (Varrh3) and the single patient with intraventricular block (Block 1) recognized the $beta_1$-peptide only. Two of the three sera from patients with primary ventricular arrhythmias (Varrh1 and Varrh2) recognized both the $beta_1$- and $beta_2$-peptides. The **middle** and **right panels** show the effect of adding, successively and without washing, a selective $beta_1$-blocker (bisoprolol) and a selective $beta_2$-blocker (ICI 118,551). See text for further discussion.

ment of such conditions and may be the starting point for many new studies.

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