Muscarinic Cholinergic Antibody in Experimental Autoimmune Myocarditis Regulates Cardiac Function (43155)

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Abstract. **Evidence is presented showing that in experimental autoimmune myocarditis, there are certain components in IgG fraction of the sera that bind to myocardium muscarinic cholinergic receptors. The autoimmune IgG simulated the biologic effect of cholinergic agonists because (i) it increased cGMP levels, (ii) it decreased cAMP stimulated levels, and (iii) it reduced heart contractility and diminished reactivity to exogenous acetylcholine. Autoimmune IgG inhibited the binding of specific muscarinic cholinergic radioligand to purified myocardial membranes behaving as noncompetitive inhibitors. The recognition appears to be organ specific because the autoimmune IgG did not bind to muscarinic cholinergic receptors of urinary bladder. The presence of antibodies against antigens expressed in an accessible form to antibody in living myocardial cells might be related to some of the immunopathologic mechanisms participating in the pathogenesis of the experimental autoimmune myocarditis.**

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The pharmacologic aspect of an immune response is a modern concept that emerges from the application of pharmacologic principles to the elucidation of immunologic events (1). Thus, an antigen could be understood as a drug switching on different and specific receptors on committed cells. Conversely, each element raised against that antigen could be seen as a high affinity and also specific pharmacologic agent.

This approach has been useful in the comprehension of diseases comprising one organ as the target of an entering antigen, namely, microbial, drug, or others, taken as models of potential autoaggression phenomena. Hence, the involvement of specific immune cells and antibodies in the impairment of various organ functions has been described (2–7), and a causal relation between severity of disease and degree of immune response has been postulated (8).

If not all, a great number of organ-localized autoimmune diseases have good correlates in animal experimental models obtained by immunization with tissues emulsified in Freund adjuvant, and this is the case with our autoimmune myocarditis model in mice, which highly resembles chagasic experimental and human disease (9). Chagas' heart disease is characterized by infiltrative lymphomononuclear myocarditis, electrocardiographic abnormalities, and striated-muscle reactive antibodies (10–13). The morphology of the chronic Chagas' heart is even more peculiar than other Chagas' syndromes and shows some features completely unknown outside the American continent. Koberle (14) has reported a neurogenic nature of this heart disease, caused by poor regulation of the autonomic control of heart activity. Denervation of both parasympathetic and sympathetic systems of the heart was verified, and it proved to be higher in the former (15).

Accordingly, the autoimmune myocarditis model presented some features resembling Chagas' disease. A similar pattern of lymphomononuclear cell infiltration, enlargement of the QRS complex, and antibodies that recognize both intracellular and surface antigens on myocardial cells were reported (9). No signs of necrosis of myocardial fibers or fibrotic lesions could be observed in this model, in contrast with chagasic experimental infection (12). We were able to demonstrate by pharmacologic means an important role of cellular infiltration on heart failure (16, 17) and humoral re-

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sponse involved in β-adrenergic regulation (18). On the other hand, this model appeared to be more advantageous than previously reported since it avoided the need of repeated antigen (Ag) inoculations (19) and did not present spontaneous heart alterations as in guinea pigs (20). Moreover, the strain of mice is the same as that used for parasitic experimental infection, creating a suitable model to analyze similarities and differences that correlate with an immunopathologic mechanism operating in Chagas' heart disease.

The aim of the present report is to present new evidences of antibody involvement in the regulation of cardiac function. We show that certain components of IgG fraction were able to bind to muscarinic cholinergic receptors, simulating the biologic effect of its agonist by increasing cGMP and decreasing cAMP, diminishing heart contractility, and reducing reactivity to exogenous muscarinic agonists.

Materials and Methods

Animals and Schedule of Immunization. BALB/c mice 45 days old from Comisión Nacional de Energia Atómica (Argentina) were used throughout. They were separated into three groups of the same age and immunized with isogenic myocardium and adjuvant (Group 1, $n = 26$), adjuvant only (Group 2, $n = 21$), or nonimmunized (Group 3, $n = 21$).

Immunization procedures were carried out as reported previously (17). Briefly, hearts were carefully removed and homogenized in phosphate-buffered saline at 4°C; tissue was filtered and half of this preparation was heated at 80°C for 15 min to enhance immunogenicity, cooled, and mixed with the other half. After protein concentration adjustment to 20 mg/ml, the preparation was emulsified with Freund's complete adjuvant (1:1) (Gibco Laboratories). The mice were given subcutaneous injections on both sides of the dorsal region with 0.4 ml of this emulsion, and a booster dose with similar characteristics was achieved 21 days later. Animals were sacrificed on Day 60. Group 2 received injections in the same way, with an emulsion consisting of 0.2 ml of phosphate-buffered saline instead of the tissue homogenate, and 0.2 ml of Freund's complete adjuvant.

Purification of IgG. Total IgG was isolated from pooled sera of each of the three groups by precipitation with 50% ammonium sulfate, followed by two or three washings and reprecipitations with 33% ammonium sulfate. After dialyzing overnight against the elution buffer, it was passed through DEAE-cellulose columns (Sigma Chemical Co.) previously equilibrated with 10 m*M* phosphate buffer (pH 8). The eluted peaks were concentrated by ultrafiltration (Minicon B15 concentrator; Amicon Corp.) to about 9–10 mg protein/ml. The degree of IgG purification was tested by microimmunoelectrophoresis, using goat anti-mouse total Ig (Cappel Laboratories). Only one line of precipitation was obtained in all cases. The titer of heart-reactive antibodies was determined by indirect immunofluorescence on mouse heart cryostat slices with a fluorescein-labeled rabbit anti-mouse Ig (Sigma). Titers for Group 1 were always higher than 1/300 showing both intracellular and surface staining. IgG from Group 1 is referred to as "autoimmune IgG," from Group 2 as "control IgG," and from Group 3 as "normal IgG."

In order to obtain a myosin-independent IgG fraction, absorption of autoimmune myocarditis sera was carried out as described previously (9). Control or autoimmune sera were incubated with bovine heart myosin (0.7 mg/ml serum) for 1 hr at 37°C followed by 48 hr at 4°C, and then IgG fraction was isolated as stated above.

Indirect Immunofluorescence Test over Primary Cultures of Myocardial Cells. The presence of circulating antibodies reactive with striated muscle was investigated by an indirect immunofluorescence technique on primary myocardial cultures of fetal rats (preterminum) (9). Cultures were performed according to the method of Simpson and Savion (21) on Leighton coverslips, and the adequate growth of myocardiocytes was confirmed by the presence of beating cells. The immunofluorescence assay was carried out by incubating cells unfixed with sera from autoimmune or control animals diluted 1/10. Heart-reactive antibodies were developed by means of a fluorescein-labeled rabbit antiserum toward mouse 7S γ-globulin (Cappel Laboratories). Readings were carried out with a Carl Zeiss microscope fitted with an epi-illuminator, with an HBO 50 mercury lamp. Micrographs were obtained with a Carl Zeiss photographic camera with an automatic photometer.

Atrial Preparations. BALB/c mice were decapitated and the auricules quickly removed and placed in a glass chamber containing a modified Krebs-Ringer-bicarbonate solution, gassed with 5% CO_2 in oxygen, maintained at pH 7.4 and 30°C. After 1 hr to reach equilibrium, control values for tension and beating frequency of isolated atria were recorded using a force transducer coupled to an ink writing oscillograph. The preparations were paced by means of a bipolar electrode using a SK4 Grass Stimulator. The stimuli had a duration of 2 msec and a voltage 10% above threshold. A constant resting tension of 350 mg was applied to atria as a preload tension. Inotropic effects (dF/dt) were assessed by recording the maximum rate of tension development during electrical stimulation at a frequency of 300 beats/min, which is close to the resting heart rate of mice, and consequently inotropy could be evaluated within the physiologic range of the force-frequency relationship. Absolute values for dF/dt were 4.4 ± 0.9 g/sec ($n = 60$) and were considered 100% dF/dt before any intervention. The heart rate was evaluated

while the atria were beating spontaneously and was expressed in beats/min.

cGMP and cAMP Determination. BALB/c mice atria were excised immediately postmortem and suspended in 3 ml of Krebs-Ringer-bicarbonate solution and gassed with 5% CO_2 in oxygen at 30°C with 5×10^{-7} *M* control and autoimmune IgG for different times in order to find the incubation period that gave the maximal effect. The effect of the IgG on cAMP was analyzed upon atria stimulated for 10 min with isoproterenol (10^{-7} *M*), whereas the effect on cGMP was explored in basal conditions. In those assays carried out in the presence of both muscarinic agents and IgG, tissues were incubated during 30 min in the case of atropine or 10 min with carbachol before and after IgG addition, respectively. The total incubation time did not exceed 40 min and at the end, atria were homogenized in 1 ml of 6% trichloroacetic acid for cGMP determination or 2 ml of ethanol for cAMP at three different settings of an Ultra-Turrax homogenizer for 15 sec each. After centrifugation at 4°C and 5000*g* for 15 min, supernatants for the cAMP assay were evaporated to dryness, whereas those for cGMP determination were extracted four times with four volumes of water-saturated diethyl ether prior to evaporation. Residues were dissolved in 300 μl of acetate buffer, and 50 or 100 μl were used for cAMP (Amersham) and cGMP (New England Nuclear) radioimmunoassay kits, respectively.

Binding Assay. Binding assays were performed in cardiac muscle and urinary bladder smooth muscle tissue, both with muscarinic type related receptors (22, 23). Membranes were prepared from above-mentioned tissues essentially as described by Limas (24). In brief, after elimination of connective tissue, fat, great vessels, and blood, atria and urinary bladder were homogenized at 4°C in six volumes of 5 m*M* Tris-HCl (pH 7.5), 1 m*M* $MgCl_2$, and 0.25 *M* sucrose in an Ultra-Turrax at settings 4, 8, and 10 for 15 sec each. The homogenate was centrifuged at 4°C and 3000*g* for 10 min twice. The pellets were discarded and the supernatants were centrifuged at 4°C and 10,000*g* for 15 min and then at 40,000*g* for 1 hr. The resulting pellets were resuspended in the incubation buffer consisting of 50 m*M* Tris-HCl (pH 7.5), 5 m*M* $MgCl_2$ (pH 7.5). Protein concentration was determined according to the method of Lowry *et al.* (25).

Binding conditions were similar to those described by Mattera *et al.* (23). Membranes were preincubated in the incubation buffer in the presence or absence of different concentrations of control or autoimmune IgG during 30 min at 30°C with shaking. IgG-treated membranes were washed twice at 4°C, resuspended in incubation buffer, and used immediately for binding assays. The membrane suspension (50–150 μg protein) and (−)-[^{3}H]quinuclidinyl benzilate ((−)-[^{3}H]QNB) (New England Nuclear), about 40 Ci/mmol, were incubated by shaking at 22°C for 60 min where equilibrium had been achieved in a final volume of 150 μl in incubation buffer. In competition studies, a fixed radioligand concentration of 0.3 n*M* and different concentrations of IgG or muscarinic agents were used. In saturation studies, IgG was included at 5×10^{-7} *M* and (−)-[^{3}H]QNB concentration range was 0.05–6 n*M*.

Chemicals. Acetylcholine, carbachol, atropine, and GTP were obtained from Sigma. The drugs were diluted to achieve final concentrations shown in the text.

Statistical Analysis. Statistical significance of differences was determined by a two-tailed *t* test for independent populations. When multiple comparisons were necessary, the Student-Newman-Keuls test was used after analysis of variance. Differences between means were considered significant at $P \leq 0.05$.

Results

Characteristics of Myocarditis in Mice Immunized with Heart Tissue. Myocarditis was assessed by histologic studies on heart slices from each group, indirect immunofluorescence to detect circulating heart reactive antibodies, and electrocardiographic studies. The histologic appearance of heart-immunized cardiac tissue was characterized by a moderate degree of inflammatory infiltrate: 91% of animals ($n = 26$) immunized with heart showed lymphomononuclear infiltrates diffuse in essence with a mean of lymphomononuclear cells (LMC) of 181 ± 25 LMC/64 cm^2 in atria and 117 ± 22 LMC/64 cm^2 in ventricles. A few focal lesions were also found.

The presence of antibodies reactive with heart antigens was stated in 24 of 26 mice immunized with cardiac tissue with an essentially sarcolemmal pattern, and the titers were always higher than 1/300 (Fig. 1). Electrocardiographic alterations consisted mainly of an enlargement of the QRS complex, and all myocarditis mice were affected. Animals from Groups 2 and 3 showed no alterations in these three parameters.

Biologic Effect of Autoimmune IgG. *Action upon mechanical activity.* Atria from normal mice were exposed to increasing concentrations of autoimmune, control, or normal IgG and a decrease in contractile parameters could be observed (Fig. 2). This effect could be blocked by atropine, suggesting a muscarinic cholinergic receptor-mediated mechanism. Neither control nor normal IgG was able to change atrial behavior. On the other hand, IgG absorption with myosin did not modify the results (IgG without absorption at 10^{-7} *M*: 2.94 ± 0.2 g/sec, $n = 12$; IgG absorbed with myosin at 10^{-7} *M*: 2.89 ± 0.3 g/sec, $n = 10$). When a subthreshold concentration of autoimmune IgG (10^{-9} *M*) was assayed in the presence of muscarinic cholinergic agonists

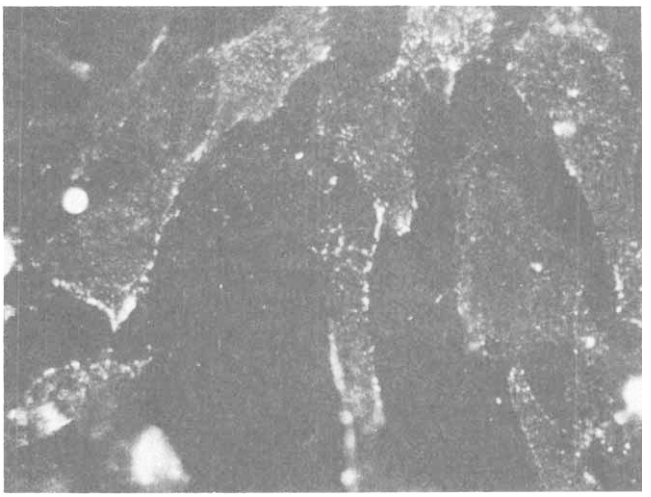

Figure 1. Indirect immunofluorescence test of autoimmune IgG (1:10) over primary cultures of embryonic rat myocardium (original magnification ×250).

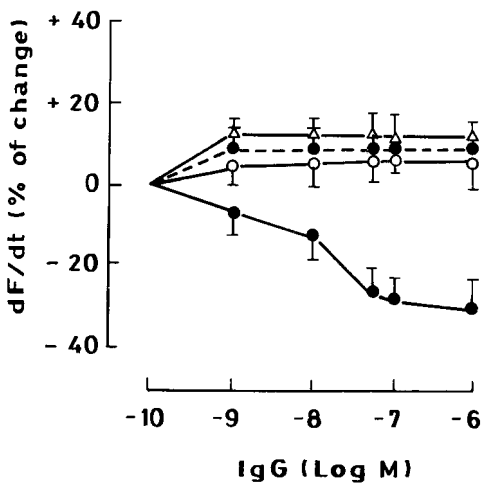

Figure 2. Effect of different concentrations of autoimmune IgG (●——●), normal IgG (○——○), and control IgG (△——△) on the dF/dt of atria. Autoimmune IgG was allowed to react for 15 min with isolated mice atria suspended in Krebs-Ringer-bicarbonate in the absence or presence of 10^{-7} *M* atropine (●- - -●). Changes in dF/dt are expressed as a percentage of variation from initial controls. Points represent mean values ± SE from six experiments in each group.

as acetylcholine and carbachol, IgG acted as a noncompetitive inhibitor of their activity (Fig. 3).

Action on intracellular cyclic nucleotide levels. In order to evaluate intracellular signals triggered by autoimmune IgG on the heart tissue, we measured changes in basal cGMP and isoproterenol-stimulated cAMP levels. Mouse hearts were exposed to autoimmune or control IgG for different times to assess IgG kinetic behavior (Fig. 4). Maximal increments induced by autoimmune IgG on cGMP, as well as cAMP decrement, were attained at 10 min and persisted at least for 20 min, being a concentration-dependent phenomenon (Fig. 4). Cyclic nucleotide levels were then measured at 10 min in further experiments with control and autoimmune IgG. Table I shows that autoimmune IgG increased 2-fold the cGMP levels above basal values, whereas it decreased cAMP-stimulated levels, and both effects could be partially blocked by 10^{-7} *M* atropine. The effect of 5.10^{-7} *M* autoimmune IgG was similar to that elicited by 10^{-7} *M* carbachol. Additionally, a subthreshold concentration of autoimmune IgG decreased the effect of the cholinergic agonist on both cyclic nucleotide levels (Table I).

Competition Binding Assay. Membranes of atrial tissue were incubated in the presence of control or autoimmune IgG with the specific muscarinic radioligand (−)-[^{3}H]QNB. Figure 5A shows the concentration-dependent inhibition of specific (−)-[^{3}H]QNB binding by autoimmune IgG, which was compared with the effect of muscarinic agents and control IgG. The inhibitory effect of IgG was time dependent because it started at 5 min, reached its maximum at 30 min, and remained constant for at least 60 min. Furthermore, (−)-[^{3}H]QNB displacement could not be achieved by the autoimmune IgG if it was added after (−)-[^{3}H]QNB had reached equilibrium (data not shown).

In order to see whether it was an organ-specific effect, a set of similar experiments was carried out on urinary bladder membranes, a tissue fraction displaying a related type of muscarinic cholinergic receptors. The results shown in Figure 5B indicate cardiac tissue specificity of autoimmune IgG.

Saturation studies with (−)-[^{3}H]QNB on cardiac membranes and Scatchard analysis of the data reflected a saturable process to a single population of noncooperative binding sites (B_{max}: 579 ± 28 fmol/mg protein) with an equilibrium K_d of 0.23 ± 0.02 n*M*. From the analysis of Scatchard plots (Table II) in the presence of autoimmune or control IgG and the calculated equilibrium parameters, it can be inferred that the IgG effect is essentially due to a change in the number of available binding sites (B_{max}; 379 ± 20 fmol/mg protein) with no significant changes in the K_d (0.21 ± 0.02 n). Interestingly, 10^{-9} *M* autoimmune IgG did not modify the equilibrium parameters, as was observed in contractility studies. Therefore, autoimmune IgG may be considered as a noncompetitive inhibitor of (−)-[^{3}H]QNB binding. Once again, experiments with autoimmune IgG previously absorbed with myosin gave a similar pattern of inhibition to that obtained with nonabsorbed autoimmune IgG.

To further characterize the nature of the interaction between this IgG and muscarinic cholinergic receptors, an experiment was done in the presence of a guanine nucleotide (GTP). In Figure 6A, it can be seen that 1 m*M* GTP was not able to modify either autoimmune or control IgG interaction. On the other hand, it can be seen in Figure 6B that GTP affected only the carbachol displacement curve but had no effect on the antagonist (atropine) behavior.

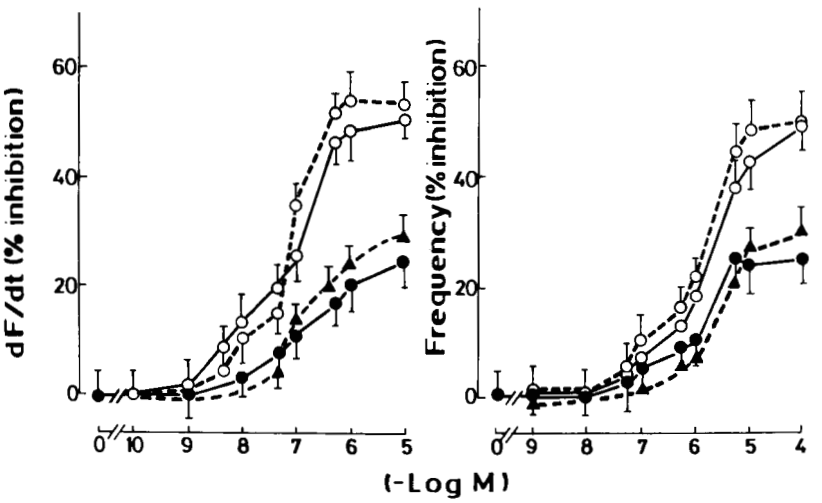

Figure 3. Inhibitory effect of autoimmune IgG on dF/dt and frequency dose-response curves of exogenous acetylcholine and carbachol. Dose-response curves of acetylcholine alone (○——○) and carbachol alone (○- - -○) or acetylcholine plus autoimmune IgG (●——●) and carbachol plus autoimmune IgG (▲- - -▲) were performed on normal mice atria. Autoimmune IgG was used at a subthreshold concentration (10^{-9} *M*). Values are mean ± SE of six experiments performed in each group.

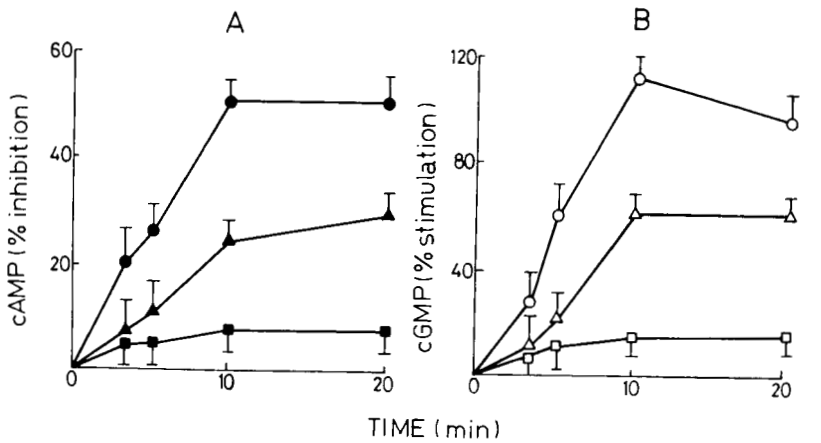

Figure 4. Time course of cAMP (A) and cGMP (B) production in the presence of 10^{-7} *M* (●, ○), 10^{-8} *M* (▲, △), or 10^{-9} *M* (■, □) autoimmune IgG. Results are expressed as percentage of variation from control IgG values in basal cGMP or 10^{-7} *M* isoproterenol-stimulated cAMP values. Neither control IgG nor normal IgG altered cyclic nucleotide levels. Values are mean ± SE of eight experiments in each group.

Discussion

Autoimmune myocarditis developed by mice given injections of cardiac antigens is a good approach to chagasic experimental and human disease (9). From a pharmacologic point of view, a number of alterations in cardiac function have been assigned to immunologic elements as specific lymphocytes and antibodies in this and other diseases in which the heart appears to be the target of the response (3, 4, 6, 17).

Here we report the effects of IgG from animals with autoimmune myocarditis on heart function. Modifications of contractile parameters could be observed that are in agreement with a muscarinic receptor-mediated effect. Stimulation of the heart muscarinic receptors reduces the contractile tension through activation of potassium channels and reduction of slow calcium influx, both effects coupled to guanine nucleotide binding proteins (26, 27). In addition to the negative inotropic effect, the occupancy of muscarinic cholinergic receptor results in increased cAMP (26, 28) and decreased cGMP formation (29), along with an increased hydrolysis of phosphoinositides (30). Beside the modification of contractile parameters, autoimmune IgG was able to decrease cAMP-stimulated levels and to increase cGMP in a time- and concentration-dependent manner, with the maximal effect of IgG being similar to that elicited by 10^{-7} *M* carbachol and partially antagonized by atropine. It was also shown that autoimmune IgG, at a concentration with no inotropic or cAMP and cGMP effect, inhibited the action of the agonist on both cyclic nucleotides. This "agonist-like antibody" would have an impaired intrinsic efficacy compared with that of a full agonist. A behavior such as the one shown by the autoimmune IgG could arise

Table I. Effect of Autoimmune IgG on Intracellular cGMP and Isoproterenol-Stimulated cAMP Levels[a]

Addition	pmol/g wet weight	
	cGMP	cAMP
None	35.0 ± 2.2	1086 ± 73
cIgG[b]	33.3 ± 2.6	984 ± 56
nIgG	34.2 ± 2.9	1109 ± 84
aIgG	84.5 ± 7.6[c]	773 ± 37[c]
aIgG + atropine	50.1 ± 3.2	873 ± 27
Carbachol	95.1 ± 3.2[c]	716 ± 41[c]
aIgG + carbachol	33.3 ± 2.4[d]	865 ± 62[d]

[a] Values are mean ± SE of six separate experiments performed in duplicate in each group. Cyclic nucleotide levels were measured in tissue homogenates after 10 min of reaction with $5 \cdot 10^{-7}$ *M* control IgG, normal IgG, autoimmune IgG, or $1 \cdot 10^{-7}$ *M* carbachol. In cAMP determinations, hearts were previously stimulated with $1 \cdot 10^{-7}$ *M* isoproterenol and then the reagents were added. The cAMP basal value before isoproterenol stimulation was 486 ± 47 pmol/g wet weight. Inhibition studies were performed incubating heart tissue with $1 \cdot 10^{-7}$ *M* atropine for 30 min before homogenizing. Autoimmune IgG when added together with carbachol was included at a subthreshold concentration ($1 \cdot 10^{-9}$ *M*).
[b] cIgG, Control IgG; nIgG, normal IgG; aIgG, autoimmune IgG.
[c] $P < 0.05$ for aIgG or carbachol versus nIgG.
[d] $P < 0.05$ for carbachol versus aIgG + carbachol.

from an allosteric interaction with the muscarinic receptor when binding to the antigens to which it was built, and would explain its dual effect depending on the concentration used. A variety of known and unknown factors influences the ability of an agonist to elicit a physiologic response. Actually, since it depends on a sequence of reactions, the final response may not be linearly related to receptor occupancy, a fact that could explain why 10^{-9} *M* autoimmune IgG was sufficient to give maximal inhibition of carbachol contractile effect while yielding slightly decreased dF/dt (Fig. 2), a situation comparable with that of a partial agonist.

The presence of a muscarinic cholinergic autoantibody was further confirmed in binding studies in which a noncompetitive interaction with the radioligand could be assessed. Those studies also served to establish the organ-specific nature of the interaction, because it resulted from the inability of autoimmune IgG to inhibit (−)-[^{3}H]QNB binding on urinary bladder membrane, a tissue known to be rich in subtype-related muscarinic cholinergic receptors (22). The myosin independence of the phenomenon was also stated in binding assays. A myosin-related autoantibody has been reported in a well-studied myocarditis like the Coxsackie virus B3-induced myocarditis in experimental models highly resembling human disease (31, 32). Moreover, a β-adrenergic effect could be found in the sera of humans with idiopathic dilated cardiomyopathy, but the myosin relationship of such autoantibodies was not tested (33). It is important to note that the autoimmune IgG, which has shown a myosin-independent character, was raised against a large number of cardiac antigens. In fact, we have reported the presence of antibodies in this experimental model that recognize antigens located both intracellularly and on the surface of the cells. The first one was related to myosin, whereas the latter, reactive against superficial antigens on primary cultures of myocardial cells, proved to be myosin independent. It is just in this surface-reactive fraction that previously reported β-adrenergic (18) and the present muscarinic cholinergic antibodies seem to be included.

The binding of muscarinic cholinergic agonists to their receptors involves a series of steps including Gi, the so-called inhibitory regulatory component of adenylate cyclase, and a guanine nucleotide effect on agonist binding affinity has been described (23, 35, 35). Carbachol in the presence of GTP was less potent to inhibit (−)-[^{3}H]QNB binding to murine atrial muscarinic cholinergic receptors, whereas atropine showed no nucleotide effect. Autoimmune IgG, on the other hand, failed to act in a nucleotide-dependent way as carbachol did, suggesting a rather complex nature of interaction, and with similar characteristics of the behavior reported for a partial agonist (36). It cannot be neglected that other antibody(ies) within this IgG preparation might interfere with the GTP ability to decrease the apparent affinity of an "agonist IgG."

Other immune sera can also bind and modulate cardiac β-adrenoceptor activity. In fact, we have already

Table II. Characteristics of (−)-[^{3}H]QNB Binding to Atrial Mouse Membranes[a]

IgG	K_d (n*M*)	B_{max} (fmol/mg protein)	No. of experiments
cIgG[b]	0.23 ± 0.02	579 ± 28	5
nIgG	0.25 ± 0.03	582 ± 30	5
aIgG (5.10^{-7} *M*)	0.21 ± 0.02	379 ± 20[c]	6
aIgG (1.10^{-9} *M*)	0.22 ± 0.03	520 ± 25	4
aIgG absorbed with myosin	0.22 ± 0.03	352 ± 15[c]	5

[a] Scatchard analysis of saturation curves for (−)-[^{3}H]QNB binding to atria membranes in the presence of 5.10^{-7} *M* control IgG, normal IgG, and autoimmune IgG absorbed or not with myosin and 0.05–6 n*M* radioligand. The incubation conditions were those described in Materials and Methods. Values are mean ± SE of the number of experiments shown performed in triplicate.
[b] cIgG, Control IgG; nIgG, normal IgG; aIgG, autoimmune IgG.
[c] $P < 0.05$ for aIgG or aIgG absorbed with myosin versus nIgG or cIgG.

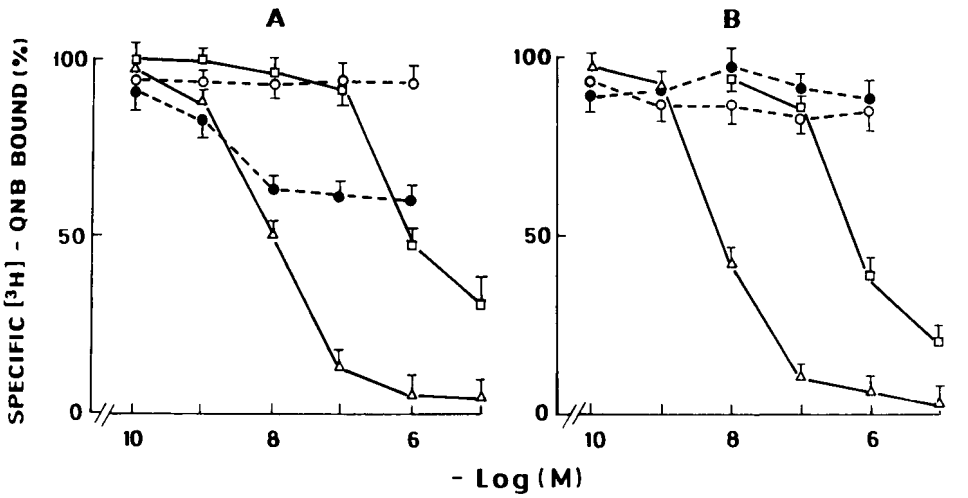

Figure 5. Inhibition of (−)-[^{3}H]QNB binding to cardiac (A) and urinary bladder (B) membranes by increasing concentrations of autoimmune IgG (●– – –●), normal IgG (○——○), or control IgG (○– – –○), atropine (△——△), or carbachol (□——□). Membranes were preincubated with different concentrations of IgG during 30 min at 30°C and then with 0.3 n*M* [^{3}H]QNB at 22°C for 60 min. Atropine and carbachol were added at the same time as the radioligand. Control binding of 100% refers to the radioactivity bound to membranes in the absence of IgG. Means of six independent experiments in each group performed in triplicate were plotted.

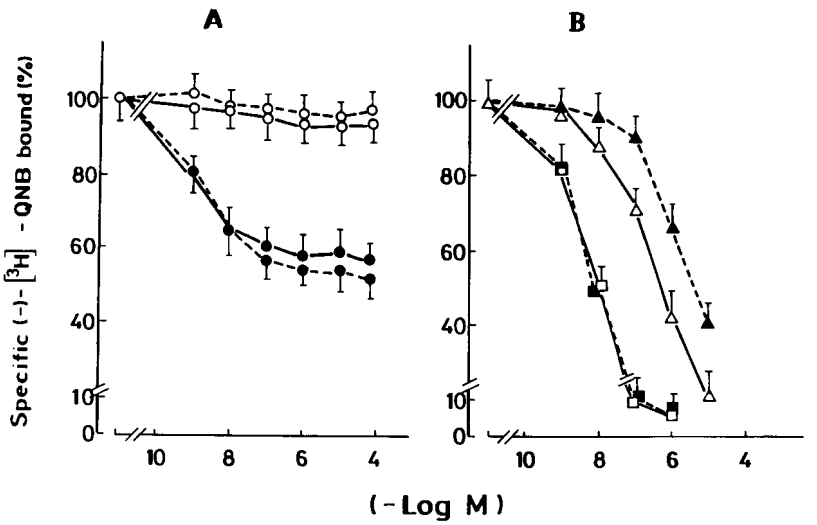

Figure 6. Inhibition of (−)-[^{3}H]QNB binding to cardiac membranes by increasing concentrations of (A) autoimmune IgG (●——●), autoimmune IgG plus GTP (●– – –●), control IgG (○——○), control IgG plus GTP (○– – –○), and (B) carbachol (△——△), carbachol plus GTP (▲– – –▲), atropine (□——□), and atropine plus GTP (■– – –■). GTP was used at 1 m*M*. Membranes were incubated with different concentrations of IgG, carbachol, or atropine during 30 min at 30°C prior to the standard (−)-[^{3}H]QNB binding assay. All assays were carried out simultaneously with membranes without any additions, and data were referred to control binding (100%). Mean ± SE of five independent experiments in each group performed in triplicate were plotted.

reported that chagasic IgG (3), alloimmune IgG (37), and autoimmune myocarditis IgG (18) are able to induce inotropic action on myocardial tissue displaying different pharmacologic behaviors. Although chagasic IgG interacted with β-adrenergic receptors in a noncompetitive manner, the same as autoimmune IgG, alloimmune IgG directed to Class I histocompatibility antigens acted as a competitive inhibitor.

The work presented here refers to the role of IgG fraction from autoimmune myocarditis sera on different parameters of cardiac function, which could influence the mechanism operating in the autoimmune myocarditis dysfunction. The demonstration of autoantibodies is of great value in the diagnosis of many common diseases, and the practical considerations arising from an animal model would allow us to gain more insight into the mechanism by which autoimmune disorders could be prevented or, at least, reduced.

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