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Effects on heart rate of an anti-M2 acetylcholine receptor immune response in mice

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▶ ABSTRACT

Autoantibodies in vitro modulating the M2 acetylcholine receptor (M2ACh-R) were observed in patients with idiopathic dilated cardiomyopathy (IDC) or Chagas' cardiomyopathy (ChC). We investigated the in vivo consequences on heart rate of such antibodies in mice immunized with a peptide derived from the second extracellular loop of the M2ACh-R compared with mice immunized with an irrelevant peptide. Sera of mice immunized with the M2ACh-R-derived peptide recognized the M2ACh-R on immunoblots and enhanced agonist activity of carbachol toward the M2AChR transfected in CHO cells. In vivo, no difference could be shown in heart rate or heart rate variability between the two groups of mice. The decrease in heart rate induced by carbachol was more pronounced, however, in the M2ACh-R immunized mice. The increase in heart rate induced by atropine, gallamine, and isoproterenol was significantly attenuated in the M2ACh-R immunized mice. Analysis of heart rate variability further argued for an increased parasympathetic response to different drugs in the M2ACh-R immunized mice. Antibodies raised against the M2AChR can behave as positive M2AChR allosteric modulators in vivo. They might be protective in boosting the activity of the parasympathetic drive to the heart, especially in patients with a high sympathetic tone.—Peter J.-C., Tugler, J., Eftekhari, P., Maurice, D., Hoebeke, J., Roegel, J.-C. Effects on heart rate of an anti-M2 acetylcholine receptor immune response in mice.

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Key Words: electrocardiography • heart rate • acetylcholine • receptors • antibodies

▶ INTRODUCTION

The M2 muscarinic receptor (M2ACh-R) belongs to the family of the G-protein-coupled receptors. It plays an important role in the regulation of the cardiac function. The parasympathetic drive to the heart is mediated via the release of acetylcholine (ACh) by the vagal nerves. Cardiac M2 muscarinic receptors (1) [E](#) via the heterotrimeric Gi/Go protein respond to this release by decreasing sinus rate, atrial contractility, and atrioventricular conduction velocity (2) [E](#). The α subunit of the Gi protein inhibits adenylyl cyclase (3) [E](#) whereas the $\beta\gamma$

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subunit of the Gi protein directly activate the inwardly rectifying muscarinic K⁺ channel (4, 5, 6).

Autoantibodies directed against the second extracellular loop of the M2ACh-R have been found in the plasma of patients with Chagas' disease (7, 8) and have been reported to behave as partial agonists in vitro (9). Hernandez et al. (10) demonstrated that the autoantibodies exert an agonist-like effect on the M2ACh-R, which may explain the bradycardia (through activation of $I_{K_{ACh}}$) and the atrioventricular blocks (through reduction of I_{Ca}) frequently associated with chronic Chagasic cardiopathy. Sera from *Trypanosoma Cruzi* infected mice were alike shown to decrease the basal Ca²⁺ current in guinea pig ventricular myocytes via muscarinic stimulation (11).

Antibodies against M2ACh-R were also found in sera of patients with idiopathic dilated cardiomyopathy (IDC) (12, 13, 14). These antibodies were able to decrease in a dose dependant manner the beating frequency of cultured neonatal rat myocytes without desensitization (14). Active immunization of rabbits with synthetic peptides derived from the second extracellular loops of the M2ACh-R and the β_1 adrenergic receptor showed a significant increase in heart weight (15). Concordant observations thus argue for the pathophysiological importance of antibodies against the second extracellular loop of the M2ACh receptor. Chiale et al. proposed that the acetylcholine-like effect of anti-M2ACh-R antibodies might play a major role in sinus node dysfunction in patients suffering from selected cardiac disorders (16). A recent study demonstrated by electrophysiological experiments that sera from IDC patients that have antibodies directed against the M2ACh-R act like agonists on the latter (17).

In this study, we aimed to demonstrate in vivo pharmacological effects of antibodies raised against the M2ACh-R in mice. The animals were immunized with a peptide corresponding to the N terminus part of the second extracellular loop of the M2ACh-R. The effects of the induced antibodies were explored in vitro on CHO cells transfected with the M2AcCh-R and in vivo on the sinus rate function. To our knowledge, this is the first study where the potential pharmacological effects of antibodies directed against the M2ACh-R were investigated in vivo.

▶ MATERIAL AND METHODS

Peptides

The M2-G19K (GVRTVEDGECYIQFFSNA(K)) peptide, corresponding to the N terminus of the second extracellular loop (residues 167-184) of the human M2 muscarinic receptor, and a control peptide (FMDV) derived from the VP1 capsid of the protein of foot and mouth disease virus (DFGSLAPRVA) (18) were synthesized using Fmoc chemistry with an automated peptide synthesizer (19). The peptides were purified by HPLC and their integrity assessed by MALDI-TOF spectrometry.

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Animals

Eighteen female BALB/c mice, 6 wk old at the start of the experiments, 20 g each, were accustomed to tail i.v. injections while being kept in individual mouse ECG boxes for 4 wk.

Immunization and induction of antibodies in mice

These mice were immunized with 30 μ g peptide in presence of methylated BSA. Priming injections were performed i.p. in complete Freund adjuvant. Two booster injections of 30 μ g peptide were given within 4 wk in incomplete Freund adjuvant. After 5 wk, a final booster injection was given. One week after the last booster injection, mice were ECG recorded. The mice were bled 1 wk before and 1 wk after each booster injection. Serum samples were tested by ELISA for the presence of anti-peptide antibodies.

Enzyme-linked immunosorbent assay

Peptides (5 μ g/mL) were coated directly in 100 mM carbonate buffer at pH 9.6 onto microtiter plates at 37°C for 1 h. After three washes with PBS-T (PBS supplemented with 0.1% (v/v) Tween 20 (Merck, Darmstad, Germany), the wells were saturated with PBS-T supplemented with BSA 1% (w/v) at 37°C for 1 h. Sera dilutions were added to the saturated microplates and incubated at 37°C for 1 h. The wells were washed four times with PBS-T and goat anti-mouse IgG (1:5000) conjugated to horseradish peroxidase (Jackson ImmunoResearch Laboratories, San Diego, CA, USA) were allowed to react at 37°C for 1 h. The wells were washed three times with PBS-T and twice with PBS before revealing antibody binding by addition of 3,3',5,5'-tetramethyl benzidine in the presence of H₂O₂.

Membrane preparation

CHO transfected with the human M2 muscarinic receptor (20) (kindly provided by Dr. M. Waelbroeck) or untransfected CHO cells were harvested in Tris 25 mM, MgCl₂ 1 mM pH 7.2, EDTA 1 mM, and sucrose 10% buffer supplemented with an anti-protease cocktail (Roche, Nutley, NJ, USA) and homogenized using a Potter homogenizer. The samples were centrifuged at 3000 g at 4°C. The pellet was resuspended and washed again for three cycles. The supernatants of these cycles were pooled and centrifuged at 124,000 g for 1 h at 4°C. The pellets were resuspended in NaH₂PO₄ 50 mM, KH₂PO₄ 50 mM and MgCl₂ pH 7.4. The protein concentration was determined using a BCA kit (Pierce, Rockford, IL, USA).

Immunoblotting

Membrane proteins were denatured 5 min by boiling in loading buffer with β-mercaptoethanol, subjected to electrophoresis on a 10% polyacrylamide gel containing SDS, then electrotransferred on nitrocellulose membrane (21). Mice sera diluted 1:2000 were applied to the membrane and revealed by using peroxidase-conjugated goat anti-mouse (Jackson) (1:5000) and ECL+ reagent (Amersham Bioscience, Arlington Heights, IL, USA).

cAMP measurement

The biochemical effects of G19K immunized mice sera on the M2 muscarinic receptor were assessed by measuring the intracellular cAMP concentration in CHO M2ACh-R transfected cells (sera of FMDV immunized mice and naive mice were tested as controls). Activation of the M2ACh-R induces a decrease of intracellular cAMP (3). Cells were seeded in 6-well culture plates 48 h before stimulation, then washed and incubated with 1 mL of Hank's balanced medium buffered with 10 mM HEPES containing 100 μM of IBMX in order to block cAMP hydrolysis. After 60 min, sera were added at different concentrations; 30 min later carbachol 30 nM and forskolin 1 μM were added. Stimulation of the cells was performed for 15 min, then the supernatant was aspirated and the reaction was stopped by adding 1 mL of boiling water. cAMP content was determined using a competitive immunoenzymatic assay (BIOTRAK cAMP, Amersham). The protein concentrations of the samples were determined using the BCA kit (Pierce). The concentration of cAMP was reported on the protein concentration, related to the number of cells/well; the results were expressed in fmol cAMP/mg protein. Results are from duplicates of two independent experiments.

Electrocardiogram recordings and drug challenges

ECG boxes (Rodent Restainers for ECG, SESAMS, Logelbach, France) have four conductive surfaces on the bottom to position the four limbs of the conscious animal. These surfaces are coated with contact gel and connected to analog amplifiers for signal amplification and filtering (0.5–30 Hz cut-off frequencies; Bessel type filter, order 2; 50 Hz notch filter (–30 dB) (SESAMS, Logelbach, France). Amplified and filtered signals are digitized at 256 Hz on a signal processor board (64 channels, 16 bit resolution) and stored in digital form on a personal computer using the recording HEM software (Notocord, Croissy sur Seine, France).

Three-lead electrocardiograms (DI, DII, DIII derivations) were recorded from the 18 conscious mice, for 50 min, on 5 consecutive days. After 10 min restraint, the animals were injected i.v. with NaCl 0.9%, isoproterenol (2mg/kg), carbachol (0.2 mg/kg), gallamine (1 mg/kg), or atropine (1 mg/kg) on days 1, 2, 3, 4, and 5, respectively. At the start of experiment, the mean of heart rate of all the animals was 599 ± 7.6 bpm.

The ECG signal was analyzed off line. The QRS complexes were identified and artifact, ectopic beats, and normal beats were recognized and annotated (ECG-Auto, EMKA Technologies, Paris, France). The heart rate (60/mean interval between the R wave peaks of adjacent normal QRS complexes) (bpm) and RR interval spectrum calculated by means of Fast Fourier Transformation (ms²) were calculated based on the beat per beat data. The total power of RR interval spectrum provides a basic quantitative evaluation of the RR variability. Power spectral density analysis provides information on how power (variance) distributes as a function of frequency. These experiments were done in accordance with institutional guidelines (ACUC approval no. 67333).

Statistics

All data are expressed as mean ± the standard error of the mean (SE). Data were analyzed using the Minitab software. To compare the variations of heart rate after drug administration, we used a 2-way ANOVA test considering time and group (FMDV or M2ACh-R).

RESULTS

Antibody titer

All the mice immunized with the G19K peptide (derived from the second extracellular loop of the M2ACh-R) developed anti-peptide antibodies (Fig. 1^a). No cross-reaction with the G19K peptide was noticed in mice immunized with the FMDV peptide (Fig. 1^b).

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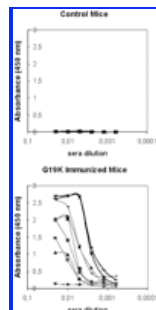


Figure 1. The presence of anti-peptide antibodies was monitored by ELISA. Sera of mice immunized with the FMDV peptide showed no response to the G19K peptide.

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Immunoblotting on membrane preparation

The ability of a pool of sera of the G19K immunized mice to recognize the M2ACh-R was tested by immunoblotting on membrane preparation from CHO human M2ACh-R transfected cells. Antibodies induced against the G19K peptide showed a specific band (Fig. 2^a) at ~55 kDa that did not appear with the control FMDV sera as well as in membrane preparation of CHO untransfected cells. The molecular weight corresponds to that of the protein revealed with an anti-M2ACh-R monoclonal antibody (22^b).

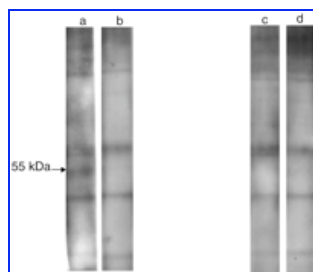
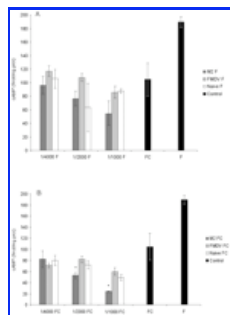


Figure 2. Immunoblots with the pooled sera from the M2-G19K immunized mice (lanes *a*, *b*) and the control FMDV immunized mice (lanes *c*, *d*) on reduced membrane protein from CHO transfected with the human M2 muscarinic receptor (lanes *a*, *c*) and untransfected CHO cells (lanes *b*, *d*). Only sera from M2-G19K immunized mice on CHO transfected cells showed a specific band at ~55 kDa corresponding to the M2 muscarinic receptor (22^b).

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cAMP measurement

Sera of the immunized animals were tested on CHO human M2ACh-R transfected cells pretreated with 1 μ M of forskolin, then the cAMP response was measured in the presence or absence of sera of the two groups and sera from naive mice. The presence of serum itself decreases the intracellular cAMP rate of treated cells. Results expressed in Fig. 3^a represent the cAMP concentration of CHO M2ACh-R transfected cells treated with FMDV or M2ACh-R sera divided by the cAMP concentration of CHO M2ACh-R transfected cells treated with sera from naive mice. Sera from the G19K immunized mice induced a decrease of intracellular cAMP in a dose-dependent manner in the presence of 30 nM carbachol (Fig. 3^b). No effect was observed with 1 μ M forskolin only compared with sera from FMDV and naive mice (data not shown). Sera from the G19K immunized mice behaved as positive allosteric modulators in CHO human M2ACh-R transfected cells.



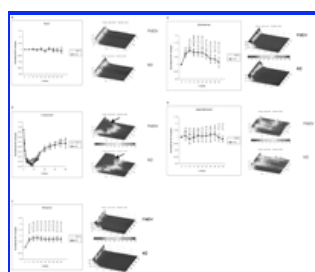
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Figure 3. Effects of sera from M2-G19K and FMDV immunized mice on cAMP in CHO M2ACh-R transfected cells treated with 1 μ M forskolin (A) or 1 μ M forskolin and carbachol 30 nM (B). Results are expressed in fmol of cAMP/mg protein. *Results of M2-G19K immunized mice sera are statistically different from those obtained with sera of FMDV immunized mice and sera of naive mice. The sera from the M2-G19K immunized mice did not have significant effects on the accumulation of cAMP compared with FMDV immunized mice and sera from naive mice on CHO M2ACh-R transfected cells pretreated with forskolin alone (A). Sera from the M2-G19K immunized mice increased, in a dose-dependent manner, inhibition by carbachol of forskolin-induced cAMP accumulation whereas sera from the control FMDV mice had no significant effect compared with sera from naive mice (B).

Effects on heart rate and drug challenges

Heart rate and RR interval spectrum were comparable in the M2-G19K and FMDV groups at baseline (mean heart rate at 622 ± 47 bpm vs. 601 ± 79 bpm; NS) as well as after administration of NaCl 0.9% (Fig. 4a, NS).



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Figure 4. Evolution of the mean heart rate \pm SE and RR interval spectrum of mice (M2-G19K immunized and FMDV control) after the different drug challenges. Heart rates were normalized for each mouse on their own value at $t = 0$. a) Heart rate and RR interval spectrum were comparable in M2-G19K and FMDV after administration of NaCl 0.9% (NS). b) Peak bradycardic effect of the muscarinic receptor agonist carbachol (0.2 mg/kg) was significantly increased ($P=0.027$) in the M2-G19K immunized mice compared with the control mice. Intensity and duration of carbachol-induced effects on RR power spectrum were obviously larger in the M2-G19K immunized mice than in control mice. The tachycardia and decreased RR variability observed after i.v. c) atropine (1 mg/kg) and d) gallamine (1 mg/kg) administration were blunted in M2-G19K immunized mice compared with control mice ($P<0.001$). e) Tachycardia and increased RR variability observed after i.v. isoproterenol (2 mg/kg) administration were equally blunted in M2-G19K immunized mice and control mice ($P<0.001$).

The peak bradycardic effect of the muscarinic receptor agonist carbachol (0.2 mg/kg) was significantly increased (for M2ACh-R immunized mice 0.38 ± 0.14 vs. 0.42 ± 0.11 , $P=0.027$; results are expressed in ratio of variation (t_i/t_0) of the heart rate before and after injection of pharmacological products at different times where t_0 is the rate at the start of injection) in M2-G19K immunized mice compared with the control mice; the extent, intensity, and duration of carbachol-induced effects on RR power spectrum were obviously larger in the M2-G19K immunized mice than in control mice (Fig. 4b).

The tachycardia and decreased RR variability observed after i.v. atropine (1 mg/kg; Fig. 4c) and gallamine (1 mg/kg; Fig. 4d) administration were blunted in the M2-G19K immunized mice compared with the control mice (respectively 1.07 ± 0.05 vs. control mice 1.14 ± 0.09 , $P<0.001$ and 1.07 ± 0.07 vs. control mice 1.18 ± 0.11 , $P<0.001$). Tachycardia and increased RR variability observed after i.v. isoproterenol (2 mg/kg) administration were equally blunted in M2-G19K immunized mice and control mice (1.00 ± 0.04 vs. control mice 1.04 ± 0.07 , $P<0.001$) (Fig. 4e).

DISCUSSION

In this study, we have evaluated the pharmacological effects of an anti-M2ACh-R immune response in vivo in mice. The animals were immunized with a peptide (M2-G19K) corresponding to the N-terminal part of the second extracellular loop of the M2ACh-R. The raised

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antibodies were able to recognize the M2ACh-R in an immunoblotting experiment. They decreased the intracellular cAMP in CHO M2ACh-R transfected cells stimulated with forskolin only in the presence of carbachol (i.e., behaved as positive allosteric modulators of the M2ACh-R in vitro). These results demonstrate quite clearly that the peptide induced functional antibodies against the M2ACh-R.

REFERENCES

The absence of partial agonist effect of the antibodies previously observed for anti-M2ACh-R antibodies from patients with cardiomyopathy on neonatal rat cardiomyocytes (14) and on rabbit cardiomyocytes (17) as well as for anti-M2ACh-R antibodies from chagasic patients on guinea pig cardiomyocytes was likely due to the type of assay. Indeed, this partial agonist effect is believed to pass through Gq and cGMP production (23), and this signaling cascade is absent in CHO M2ACh-R transfected cells.

The positive allosteric effects on the M2ACh-R were confirmed in vivo when studying heart rate and heart rate variability. To prevent additional stress, all animals were habituated to an ECG box and to tail i.v. injection for 4 wk. The animals were conscious when tested to prevent any ECG modification by anesthesia (24). Under these conditions, heart rate and RR interval spectra were comparable in the M2-G19K and FMDV groups at baseline as well as after administration of NaCl 0.9% (Fig. 4a). If the antibodies exerted a positive allosteric effect on the M2ACh-R, bradycardia would be expected. The absence of bradycardia with immunized mice could be due to a homeostatic effect, decreasing the baseline parasympathetic drive and/or increasing the baseline sympathetic drive to compensate.

In our experimental conditions, the basal parasympathetic tone was probably low in the mice immunized against the M2-G19K peptide compared with the control mice; in fact, the tachycardia and decreased RR variability observed after the administration of the muscarinic receptor antagonist atropine (1 mg/kg) and the M2ACh-R negative allosteric ligand gallamine (1 mg/kg) were blunted in the M2-G19K immunized mice compared with control mice (Fig. 4d). Gallamine has its allosteric binding site on the second extracellular loop of the M2ACh-R (25). In contrast to atropin, gallamine may compete for the same binding site as the antibodies on the M2ACh-R.

Nevertheless, the positive allosteric modulation of the receptor was evident in the presence of the ACh-R agonist carbachol. Indeed, the resulting bradycardic effect was significantly higher in mice with the M2ACh-R anti-receptor antibodies than in the control mice. The small difference in absolute heart rate values between the two groups is due to the fact that the dose of carbachol used was nearing the maximally possible parasympathetic stimulus. Moreover, duration and intensity on the RR power spectrum were prolonged. This could be due to the lack of desensitization of the M2ACh-R complexed with antibodies, as shown in vitro (14). It cannot be excluded, however, that receptor stimulation over a longer period could induce internalization phenomena as observed with chagasic sera (26).

The results obtained with the β agonist isoproterenol also favor the hypothesis of a positive modulation of the M2ACh-R. The tachycardia and increased RR variability observed after isoproterenol administration were blunted in M2-G19K immunized mice compared with control mice (Fig. 4e). The positive allosteric effect, shown by the antibodies in vitro, increased the parasympathetic response to the sympathetic stimulus, resulting in a blunted heart rate.

What is the relevance of our results for the pathological conditions in which the presence of anti-M2 ACh-R autoantibodies was described? Autoantibodies against the M2 ACh-R have been observed in cardiomyopathies in which the presence of autoantibodies against the β_1 -adrenoceptor have been demonstrated. The simultaneous occurrence of both types of autoantibodies varies from 25 to 80% (8, 13, 27). The pathogenicity of the anti- β_1 adrenoceptor autoantibodies are now well attested in view of the amelioration of heart function in dilated cardiomyopathy patients by specific immunoadsorption of such autoantibodies (28) and by recent experimental evidence that rat anti- β_1 adrenoceptor antibodies can induce cardiomyopathic changes by passive transfer (29). The pathogenic properties of anti- β_1 adrenoceptor autoantibodies have been ascribed to their potency to continuously stimulate the sympathetic drive, since the autoantibodies inhibit receptor desensitization (30). In contrast to Chagas' cardiomyopathy, in which sinus node dysfunction and bradycardia can be correlated with a dominant anti-M2ACh-R response (31), dilated cardiomyopathy has a dominant anti- β_1 adrenoceptor response, although an anti-M2ACh-R response was found in 43% of patients (17). The presence of anti-M2 AChR autoantibodies in DCM could be an example of immunological homeostasis in which the activity of anti-M2ACh-R antibodies, as described here, could compensate for the continuous sympathetic drive induced by the anti- β_1 adrenoceptor autoantibodies. The hypothesis of a beneficial autoimmune response

to counter a deleterious immune or inflammatory response has recently been proposed (32). Studies correlating the diagnostic and prognostic value of the presence of anti-M2ACh-R autoantibodies in patients with dilated cardiomyopathy positive for anti- β_1 adrenoceptor autoantibodies could in firm or confirm this hypothesis.

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- Fisher, J. T., Vincent, S. G., Gomeza, J., Yamada, M., Wess, J. (2004) Loss of vagally mediated bradycardia and bronchoconstriction in mice lacking M2 or M3 muscarinic acetylcholine receptors. *FASEB J.* **18**,711-713 [[Abstract/Free Full Text](#)]
- Luetje, C. W., Tietje, K. M., Christian, J. L., Nathanson, N. M. (1988) Differential tissue expression and developmental regulation of guanine nucleotide binding regulatory proteins and their messenger RNAs in rat heart. *J. Biol. Chem.* **263**,13357-13365 [[Abstract/Free Full Text](#)]
- Sunahara, R. K., Dessauer, C. W., Gilman, A. G. (1996) Complexity and diversity of mammalian adenylyl cyclases. *Annu. Rev. Pharmacol. Toxicol.* **36**,461-480 [[CrossRef](#)] [[Medline](#)]
- Logothetis, D. E., Kurachi, Y., Galper, J., Neer, E. J., Clapham, D. E. (1987) The beta gamma subunits of GTP-binding proteins activate the muscarinic K⁺ channel in heart. *Nature (London)* **325**,321-326 [[CrossRef](#)] [[Medline](#)]
- Sowell, M. O., Ye, C., Ricupero, D. A., Hansen, S., Quinn, S. J., Vassilev, P. M., Mortensen, R. M. (1997) Targeted inactivation of α 2 or α 3 disrupts activation of the cardiac muscarinic K⁺ channel, IK⁺ACh, in intact cells. *Proc. Natl. Acad. Sci. USA* **94**,7921-7926 [[Abstract/Free Full Text](#)]
- Yamada, M., Inanobe, A., Kurachi, Y. (1998) G protein regulation of potassium ion channels. *Pharmacol. Rev.* **50**,723-760 [[Abstract/Free Full Text](#)]
- Goin, J. C., Leiros, C. P., Borda, E., Sterin-Borda, L. (1997) Interaction of human chagasic IgG with the second extracellular loop of the human heart muscarinic acetylcholine receptor: functional and pathological implications. *FASEB J.* **11**,77-83 [[Abstract](#)]
- Elies, R., Ferrari, I., Wallukat, G., Lebesgue, D., Chiale, P., Elizari, M., Rosenbaum, M., Hoebcke, J., Levin, M. J. (1996) Structural and functional analysis of the B cell epitopes recognized by anti-receptor autoantibodies in patients with Chagas' disease. *J. Immunol.* **157**,4203-4211 [[Abstract](#)]
- Goin, J. C., Perez Leiros, C., Borda, E., Sterin-Borda, L. (1994) Modification of cholinergic-mediated cellular transmembrane signals by the interaction of human chagasic IgG with cardiac muscarinic receptors. *Neuroimmunomodulation* **1**,284-291 [[Medline](#)]
- Hernandez, C. C., Barcellos, L. C., Gimenez, L. E., Cabarcas, R. A., Garcia, S., Pedrosa, R. C., Nascimento, J. H., Kurtenbach, E., Masuda, M. O., Campos de Carvalho, A. C. (2003) Human chagasic IgGs bind to cardiac muscarinic receptors and impair L-type Ca²⁺ currents. *Cardiovasc. Res.* **58**,55-65 [[CrossRef](#)] [[Medline](#)]
- Mijares, A., Verdot, L., Peineau, N., Vray, B., Hoebcke, J., Argibay, J. (1996) Antibodies from Trypanosoma cruzi infected mice recognize the second extracellular loop of the beta 1-adrenergic and M2-muscarinic receptors and regulate calcium channels in isolated cardiomyocytes. *Mol. Cell. Biochem.* **163-164**,107-112 [[CrossRef](#)] [[Medline](#)]
- Fu, L. X., Magnusson, Y., Bergh, C. H., Liljeqvist, J. A., Waagstein, F., Hjalmarson, A., Hoebcke, J. (1993) Localization of a functional autoimmune epitope on the muscarinic acetylcholine receptor-2 in patients with idiopathic dilated cardiomyopathy. *J. Clin. Invest.* **91**,1964-1968
- Zhang, L., Hu, D., Li, J., Wu, Y., Liu, X., Yang, X. (2002) Autoantibodies against the myocardial beta1-adrenergic and M2-muscarinic receptors in patients with congestive heart failure. *Chin. Med. J. (England)* **115**,1127-1131
- Wallukat, G., Fu, H. M., Matsui, S., Hjalmarson, A., Fu, M. L. (1999) Autoantibodies against M2 muscarinic receptors in patients with cardiomyopathy display non-desensitized agonist-like effects. *Life Sci.* **64**,465-469 [[CrossRef](#)] [[Medline](#)]
- Matsui, S., Fu, M. L., Hayase, M., Katsuda, S., Yamaguchi, N., Teraoka, K., Kurihara, T., Takekoshi, N. (1999) Active immunization of combined beta1-adrenoceptor and M2-muscarinic receptor peptides induces cardiac hypertrophy in rabbits. *J. Card. Fail.* **5**,246-254 [[CrossRef](#)] [[Medline](#)]
- Chiale, P. A., Ferrari, I., Mahler, E., Vallazza, M. A., Elizari, M. V., Rosenbaum, M. B., Levin, M.

- J. (2001) Differential profile and biochemical effects of antiautonomic membrane receptor antibodies in ventricular arrhythmias and sinus node dysfunction. *Circulation* **103**,1765-1771[[Medline](#)]
17. Del Corso, C., Campos De Carvalho, A. C., Martino, H. F., Varanda, W. A. (2004) Sera from patients with idiopathic dilated cardiomyopathy decrease I-type calcium currents in cardiomyocytes isolated from rabbits. *Am. J. Physiol.* **287**,H1928-H1936
 18. Brown, F., Benkirane, N., Limal, D., Halimi, H., Newman, J. F., Van Regenmortel, M. H., Briand, J. P., Muller, S. (1999) Delineation of a neutralizing subregion within the immunodominant epitope (GH loop) of foot-and-mouth disease virus VP1 which does not contain the RGD motif. *Vaccine* **18**,50-56[[CrossRef](#)][[Medline](#)]
 19. Neimark, J., Briand, J. P. (1993) Development of a fully automated multichannel peptide synthesizer with integrated TFA cleavage capability. *Pept. Res.* **6**,219-228[[Medline](#)]
 20. Buckley, N. J., Bonner, T. I., Buckley, C. M., Brann, M. R. (1989) Antagonist binding properties of five cloned muscarinic receptors expressed in CHO-K1 cells. *Mol. Pharmacol.* **35**,469-476[[Abstract](#)]
 21. Peter, J. C., Eftekhari, P., Billiard, P., Wallukat, G., Hoebeke, J. (2003) scFv single chain antibody variable fragment as inverse agonist of the beta2-adrenergic receptor. *J. Biol. Chem.* **278**,36740-36747[[Abstract/Free Full Text](#)]
 22. Elies, R., Fu, L. X., Eftekhari, P., Wallukat, G., Schulze, W., Granier, C., Hjalmarsen, A., Hoebeke, J. (1998) Immunochemical and functional characterization of an agonist-like monoclonal antibody against the M2 acetylcholine receptor. *Eur. J. Biochem.* **251**,659-666[[Medline](#)]
 23. Nascimento, J. H., Salle, L., Hoebeke, J., Argibay, J., Peineau, N. (2001) cGMP-mediated inhibition of cardiac L-type Ca(2+) current by a monoclonal antibody against the M(2) ACh receptor. *Am. J. Physiol.* **281**,C1251-C1258
 24. Chaves, A. A., Dech, S. J., Nakayama, T., Hamlin, R. L., Bauer, J. A., Carnes, C. A. (2003) Age and anesthetic effects on murine electrocardiography. *Life Sci.* **72**,2401-2412[[CrossRef](#)][[Medline](#)]
 25. Leppik, R. A., Miller, R. C., Eck, M., Paquet, J. L. (1994) Role of acidic amino acids in the allosteric modulation by gallamine of antagonist binding at the m2 muscarinic acetylcholine receptor. *Mol. Pharmacol.* **45**,983-990[[Abstract](#)]
 26. Leiros, C. P., Sterin-Borda, L., Borda, E. S., Goin, J. C., Hosey, M. M. (1997) Desensitization and sequestration of human m2 muscarinic acetylcholine receptors by autoantibodies from patients with Chagas' disease. *J. Biol. Chem.* **272**,12989-12993[[Abstract/Free Full Text](#)]
 27. Matsui, S., Fu, M. L., Hayase, M., Katsuda, S., Yamaguchi, N., Teraoka, K., Kurihara, T., Takekoshi, N. (2001) Beneficial effect of muscarinic-2 antagonist on dilated cardiomyopathy induced by autoimmune mechanism against muscarinic-2 receptor. *J. Cardiovasc. Pharmacol.* **38**(Suppl. 1),S43-S49
 28. Wallukat, G., Muller, J., Hetzer, R. (2002) Specific removal of beta1-adrenergic autoantibodies from patients with idiopathic dilated cardiomyopathy. *N. Engl. J. Med.* **347**,1806[[Free Full Text](#)]
 29. Jahns, R., Boivin, V., Hein, L., Triebel, S., Angermann, C. E., Ertl, G., Lohse, M. J. (2004) Direct evidence for a beta 1-adrenergic receptor-directed autoimmune attack as a cause of idiopathic dilated cardiomyopathy. *J. Clin. Invest.* **113**,1419-1429[[CrossRef](#)][[Medline](#)]
 30. Magnusson, Y., Wallukat, G., Waagstein, F., Hjalmarsen, A., Hoebeke, J. (1994) Autoimmunity in idiopathic dilated cardiomyopathy. Characterization of antibodies against the beta 1-adrenoceptor with positive chronotropic effect. *Circulation* **89**,2760-2767[[Medline](#)]
 31. Chiale, P. A., Ferrari, I. (2001) Autoantibodies in Chagas' cardiomyopathy and arrhythmias. *Autoimmunity* **34**,205-210[[Medline](#)]
 32. Nevo, U., Golding, I., Neumann, A. U., Schwartz, M., Akselrod, S. (2004) Autoimmunity as an immune defense against degenerative processes: a primary mathematical model illustrating the bright side of autoimmunity. *J. Theor. Biol.* **227**,583-592[[CrossRef](#)][[Medline](#)]

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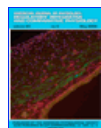
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