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Activating Autoantibodies to the Beta-1 Adrenergic and M2 Muscarinic Receptors Facilitate Atrial Fibrillation in Patients With Graves' Hyperthyroidism

Stavros Stavrakis, MD,*† Xichun Yu, MD,*†‡ Eugene Patterson, PHD,†§ Shijun Huang, MD,*†‡
Sean R. Hamlett, MD,*‡ Laura Chalmers, MD,*‡ Reji Pappy, MD,*
Madeleine W. Cunningham, PHD,‖ Syed A. Morshed, PHD,¶ Terry F. Davies, MD,¶
Ralph Lazzara, MD,*† David C. Kem, MD*†‡

Oklahoma City, Oklahoma; and New York, New York

Objectives We studied activating autoantibodies to beta-1 adrenergic receptors (AAβ1AR) and activating autoantibodies to M2 muscarinic receptors (AAM2R) in the genesis of atrial fibrillation (AF) in Graves' hyperthyroidism.

Background Atrial fibrillation frequently complicates hyperthyroidism. Both AAβ1AR and AAM2R have been described in some patients with dilated cardiomyopathy and AF. We hypothesized that their copresence would facilitate AF in autoimmune Graves' hyperthyroidism.

Methods Immunoglobulin G purified from 38 patients with Graves' hyperthyroidism with AF (n = 17) or sinus rhythm (n = 21) and 10 healthy control subjects was tested for its effects on isolated canine Purkinje fiber contractility with and without atropine and nadolol. Immunoglobulin G electrophysiologic effects were studied using intracellular recordings from isolated canine pulmonary veins. Potential cross-reactivity of AAβ1AR and AAM2R with stimulating thyrotropin receptor (TSHR) antibodies was evaluated before and after adsorption to Chinese hamster ovary cells expressing human TSHRs using flow cytometry and enzyme-linked immunosorbent assays.

Results The frequency of AAβ1AR and/or AAM2R differed significantly between patients with AF and sinus rhythm (AAβ1AR = 94% vs. 38%, $p < 0.001$; AAM2R = 88% vs. 19%, $p < 0.001$; and AAβ1AR+AAM2R = 82% vs. 10%, $p < 0.001$). The copresence of AAβ1AR and AAM2R was the strongest predictor of AF (odds ratio: 33.61, 95% confidence interval: 1.17 to 964.11, $p = 0.04$). Immunoglobulin G from autoantibody-positive patients induced hyperpolarization, decreased action potential duration, enhanced early afterdepolarization formation, and facilitated triggered firing in pulmonary veins by local autonomic nerve stimulation. Immunoadsorption studies showed that AAβ1AR and AAM2R were immunologically distinct from TSHR antibodies.

Conclusions When present in patients with Graves' hyperthyroidism, AAβ1AR and AAM2R facilitate development of AF.
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Hyperthyroidism has been associated with atrial tachyarrhythmias (1–3) and with sustained atrial fibrillation (AF) occurring in 20% to 30% of patients even after return to the euthyroid state (1,2). The pathogenesis of AF in these patients is postulated to result from shortening of the action potential duration in the atrial myocardium from excess thyroid hormone facilitating formation of multiple re-entry circuits (4,5). Graves' disease is one of the most common causes of hyperthyroidism (6). The prevalence of AF in patients with Graves' disease, as in all other forms of hyperthyroidism, increases with age (1,2,6).

The autoimmune pathogenesis of Graves' disease is accepted and attributed to autoantibodies that activate the

From the *Department of Medicine, University of Oklahoma Health Sciences Center and Veterans Affairs Medical Center, Oklahoma City, Oklahoma; †Heart Rhythm Institute, ‡Harold Hamm Oklahoma Diabetes Center, and the Departments of §Cell Biology and ‖Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma; and the ¶Thyroid Research Unit, Mount Sinai School of Medicine, James J. Peters Veterans Affairs Medical Center, New York, New York. Supported by the American Heart Association, Presbyterian Health Foundation (Dr. Yu), Heart Rhythm Institute, University of Oklahoma Health Sciences Center and the Oklahoma City Veterans Administration Research Foundation (Drs. Patterson and Kem), National Institute of Diabetes and Digestive and Kidney Diseases (DK06973), and the Veterans Affairs Merit Award program (Dr. Davies). Private grants from Will and Helen Webster, Britani T. and Paul E. Bowman, Jr., and Stan and Gayle Ward were gratefully received. Dr. Cunningham has been a consultant for Wyeth Vaccines, Aventis-Pasteur, ID Biomedical, Shire Biologics, Novartis, and Talecris. Dr. Davies has served as a board member for Kronus Corporation. This paper was presented in part at the Annual Meeting of the American College of Cardiology, March 2008, Chicago, Illinois; and at the Annual Meeting of the Endocrine Society, June 2008, San Francisco, California.

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Abbreviations and Acronyms

AAβ1AR = activating autoantibodies to beta-1 adrenergic receptor

AAM2R = activating autoantibodies to M2 muscarinic receptor

AF = atrial fibrillation

CHO-TSHR = Chinese hamster ovary cells expressing full-length thyrotropin receptor

CI = confidence interval

DT = deceleration time of mitral E flow velocity

E = early diastolic velocity of mitral inflow

E/E′ = ratio between the early diastolic velocity of mitral inflow and that of mitral annulus

ELISA = enzyme-linked immunosorbent assay

Ig = immunoglobulin

M2R = M2 muscarinic receptor

TSHR = thyrotropin receptor

G protein-coupled thyrotropin receptor (TSHR) (6,7). Activating autoantibodies to the beta-1 adrenergic receptors (AAβ1AR) and the M2 muscarinic receptors (AAM2R) variably occur in patients with several cardiomyopathies and in a subset of patients with AF (8–13). The AAβ1AR show positive inotropic and chronotropic effects (14,15), whereas AAM2R have negative chronotropic effects (13) and decrease the action potential duration in isolated cardiomyocytes (10). The presence of AAM2R was associated with the occurrence of AF in patients with idiopathic dilated cardiomyopathy (13). Combined sympathetic and parasympathetic stimulation has been shown to generate early afterdepolarizations and rapid triggered firing in the pulmonary veins, which in turn induces AF (16,17). Given the synergistic role of sympathetic and parasympathetic activity for initiation and/or maintenance of AF (18,19), we hypothesized: 1) patients with Graves' hyperthyroidism develop significant titers of AAβ1AR and AAM2R; and 2) these autoantibodies facilitate development of AF.

Methods

Study patients. Thirty-eight patients with Graves' hyperthyroidism with AF (n = 17) or sinus rhythm (n = 21) were included in the study through referral and were seen by an endocrinologist and cardiologist. The diagnosis of Graves' hyperthyroidism was based on markedly suppressed serum thyrotropin concentrations, elevated serum free thyroxine and triiodothyronine concentrations, and evidence of diffuse goiter with increased 24-h radionuclide uptake (6). Measurement of TSHR antibodies was generally obtained but not required unless there was ambiguity in the diagnosis. All patients were seen during a 2-year period. The AF was confirmed by a 12-lead electrocardiogram. Echocardiograms were performed in all but 4 patients (1 with AF and 3 with sinus rhythm). Serum was obtained from each patient and 10 voluntary healthy donors (mean age 29.5 ± 3.2 years). This study was approved by the University of Oklahoma Health Sciences Center Institutional Review Board, and all subjects provided written informed consent.

Purification of immunoglobulin (Ig) G antibody. The IgG was purified using the NAb Protein A/G Spin Kit (Pierce, Rockford, Illinois), according to the manufacturer's protocol.

Contractility bioassay. Free-running canine Purkinje fibers (5 to 7 mm) were transferred to a 36°C ± 0.1°C perfusion chamber mounted on the stage of an inverted microscope (Olympus America Inc., Melville, New York) (20). The fibers were perfused with normal Tyrode solution (in mmol/l: NaCl 145, KCl 4.5, $CaCl_2$ 1.8, $MgCl_2$ 1, NaH_2PO_4 1, glucose 11, HEPES 10, pH 7.36) at 36 ± 0.1°C and paced with a 4-ms duration constant current pulse at 2 Hz via extracellular platinum electrodes. Isometric contractions were recorded before, during steady state, and after the washout using a video edge detector (Model VED-205, Crescent Electronics, Sandy, Utah). After achieving stable contractile responses over 3 to 5 min, IgG equivalent to a 1:100 serum dilution from a patient or control subject was administered for a 5-min interval. With subsequent 5-min periods, IgG plus atropine (100 nmol/l) or nadolol (100 nmol/l) was assayed to determine the effect attributable to the AAβ1AR or AAM2R components of IgG, respectively. Isoproterenol (10 nmol/l) served as a positive control. The IgG from healthy donors served as negative control subjects. Contractility was calculated as the mean of 15 consecutive contraction cycles after a stable baseline or response was elicited and analyzed offline using pClamp 9.2 (Axon Instruments, Foster City, California). Any response that was significantly different from the baseline with a $p < 0.05$ was considered to be positive. Increased contractility over baseline with IgG plus atropine represented the AAβ1AR effect. The change in IgG effect on contractility with and without atropine was a surrogate marker of the AAM2R inhibitory effect. The intra-assay and interassay coefficients of variation were 6.6% (n = 24) and 8.6% (n = 38), respectively.

Electrical recordings. Isolated canine pulmonary vein preparations (16) were pinned endocardial side up and superfused with oxygenated Tyrode solution at 36°C (20 ml/min). A bipolar electrode recording (0.10 mm diameter Teflon-coated silver wires, 1 mm apart) was obtained, filtered at 10 to 10,000 Hz, and recorded on a Gould WindowGraf recorder (Gould Inc., Valley View, Ohio). An intracellular recording was obtained using a glass microelectrode with an intracellular resistance of 10 to 30 MΩ (Duo 773 electrometer, World Precision Instruments, Sarasota, Florida) and was maintained for the duration of evaluation of a single IgG sample. The preparation was paced at 2× to 3× diastolic threshold using 4-ms-duration stimuli from a Grass model S88 stimulator (Quincy, Massachusetts) at 1 Hz. Intracellular recordings were performed before and after autonomic nerve stimulation from the immediate vicinity of the stimulating electrodes (within 2 to 3 mm) and before and after superfusion of the preparation with IgG (0.15 mg/ml). Local autonomic nerve stimulation was accomplished using 300-ms-duration high-frequency (100 Hz) trains of 0.05-ms-duration square-wave stimuli introduced at 10 to 150 V in 20-V steps from a Grass stimulator. Voltage was maintained at <50% of the threshold voltage

required to excite local myocardium when introduced as 0.05-ms-duration stimuli during a 2-Hz pacing train.

Detection of TSHR antibodies by flow cytometry. Purified IgG samples were diluted (1:200) with isotonic phosphate-buffered saline containing 4% bovine serum albumin and 0.01% sodium azide and incubated with Chinese hamster ovary cells expressing full-length human thyrotropin receptor (CHO-TSHR cells) (21). Antihuman IgG (H+L) conjugated with fluorescein isothiocyanate (BD Bioscience Pharmingen, San Diego, California) was the secondary antibody. The mean fluorescent intensity was measured by flow cytometry (BD Bioscience Pharmingen). A human monoclonal antibody (M22) to the TSHR that stimulates cyclic adenosine monophosphate in CHO-TSHR cells confirmed TSHR-specific binding.

Adsorption study. The CHO-TSHR cells were maintained in Ham's F12 medium supplemented with 10% fetal bovine serum (Mediatech, Manassas, Virginia), 100 U/ml penicillin, and 100 U/ml streptomycin (Invitrogen, Grand Island, New York). Fully confluent cells were detached by phosphate-buffered saline containing 1 mM ethylenediaminetetraacetic acid and 1 mM ethyleneglycotetraacetic acid. Counted (1×10^6) cells were incubated with 100 μl of diluted (1:200) purified IgG for 30 min with mild rocking at room temperature. The IgG-adsorbed samples were collected by centrifugation. Flow cytometry was performed using pre- and post-adsorption samples in parallel. A reduction in mean fluorescent intensity of >25% indicated significant adsorption. All experiments were performed twice. Pre- and post-adsorbed serum samples were analyzed in duplicate by enzyme-linked immunosorbent assay (ELISA) for antibody titers to the β1AR and M2R using whole receptors expressed in membranes (PerkinElmer, Waltham, Massachusetts) (20).

Statistical analysis. Data are expressed as mean ± SD. Contractility values were normalized to their baseline values. Comparison between 2 groups was performed by using the unpaired or paired Student *t* test for quantitative variables, as applicable, and the Fisher exact test for dichotomous variables. The McNemar test was used for the matched analysis. Linear correlation was performed to examine the strength of the linear relationship between the true AAM2R effect and its surrogate. Repeated-measures analysis of variance was used to determine differences within a group with drug treatment. Logistic regression analysis was used to assess predictors of AF. Those variables with p values <0.10 by univariate analysis were included in the multivariate logistic regression analysis model, and the respective odds ratios (ORs) were calculated. All analyses were 2-tailed. Statistical significance was set at $p < 0.05$.

Results

Patient characteristics. Seventeen patients had AF and 21 had sinus rhythm. The clinical, echocardiographic, and biochemical characteristics of the patients are summarized in Table 1. Patients with AF were older than patients with sinus rhythm (60.9 ± 12.7 years vs. 45.7 ± 13.1 years, $p < 0.001$). Otherwise, no difference was noted for the percentage of male sex, presence of hypertension, diabetes mellitus, coronary artery disease, and congestive heart failure between the 2 groups. Echocardiographic indexes, including left ventricular ejection fraction, left atrial diameter, early diastolic velocity of mitral inflow (E), deceleration time of mitral E flow velocity (DT), and the ratio between the early diastolic velocity of mitral inflow and that of mitral annulus (E/E′) did not differ significantly between the 2 groups. Serum thyrotropin and free thyroxine concentrations were similar in the 2 groups.

Table 1 Clinical, Echocardiographic, and Biochemical Characteristics of the Patients

	Atrial Fibrillation (n = 17)	Normal Sinus Rhythm (n = 21)	p Value
Age (yrs)	60.9 ± 12.7	45.7 ± 13.1	0.001*
Male (%)	47.1	42.9	1.00
Hypertension (%)	70.6	61.9	0.73
Diabetes mellitus (%)	35.3	23.8	0.49
Coronary artery disease (%)	17.6	9.5	0.64
Heart failure (%)	52.9	23.8	0.09
Ejection fraction (%)	51.6 ± 16.1	52.4 ± 16.2	0.88
Left atrium (mm)	42.3 ± 7.8	38.3 ± 7.6	0.15
E (m/s)	0.83 ± 0.25	0.98 ± 0.37	0.17
DT (ms)	245.2 ± 72.2	242.4 ± 77.9	0.90
E/E′	5.9 ± 2.5	6.6 ± 2.5	0.50
Serum thyrotropin (mU/l)	0.075 ± 0.12	0.071 ± 0.18	0.94
Serum free thyroxine (ng/dl)	2.35 ± 1.3	3.08 ± 2.3	0.22

DT = deceleration time of mitral E flow velocity; E = early diastolic velocity of mitral inflow; E/E′ = ratio between the early diastolic velocity of mitral inflow (E) and that of mitral annulus (E′).

Contractility bioassay. Twenty-four (63%) and 19 (50%) of the 38 IgG samples showed AAβ1AR and AAM2R, respectively. In 16 (42%) IgG samples, AAβ1AR and AAM2R coexisted. None of 10 control IgG samples showed either autoantibody group. The β-adrenergic receptor agonist isoproterenol (10 nmol/l) increased contractility 48.4 ± 4.9% over baseline ($p < 0.001$). The mean IgG agonist effect (percent over baseline) from the 24 AAβ1AR-positive patients in the presence of M2R blockade was 25.6 ± 9.6% ($p < 0.001$ vs. control subjects). The absolute mean inhibitory AAM2R effect (percent change over baseline) in the 19 AAM2R-positive patients was 28.1 ± 16.6% ($p < 0.001$ vs. control subjects) (Fig. 1A). The change in IgG effect on contractility with and without atropine correlated strongly with the IgG effect in the presence of nadolol ($R^2 = 0.67$, $p = 0.001$, $n = 12$), supporting the use of the atropine-induced change as a surrogate for the AAM2R effect. During each assay, the effect of combined βAR and M2R blockade with nadolol and atropine led to a return of the IgG response to baseline. These data, not shown, provide additional evidence against the copresence of additional autoantibodies causing Purkinje contractile response.

The frequency of autoantibody positivity differed significantly between the 2 groups (Fig. 1B). Sixteen of 17 (94%) patients with AF were positive for AAβ1AR, compared

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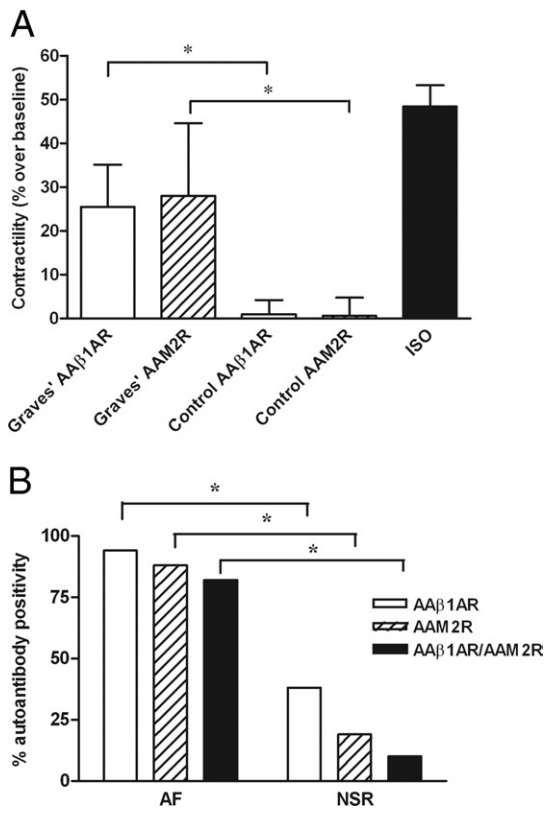

Figure 1 **Functional Effects of IgG From Patients With Graves' Disease and Control Subjects on Purkinje Fiber Contractility**

(A) The mean effect (percent over baseline) of activating autoantibodies to beta-1 adrenergic receptor (AAβ1AR) from the 24 AAβ1AR-positive patients (Graves' AAβ1AR) and the absolute value of the mean inhibitory effect (percent change over baseline) of activating autoantibodies to M2 muscarinic receptor (AAM2R) in the 19 AAM2R-positive patients (Graves' AAM2R) are compared with control immunoglobulin (Ig)G. The antibody-negative Graves' values were not significantly different from the control subjects and therefore are not shown. Isoproterenol (ISO) (10 nmol/l) served as a positive control. Both AAβ1AR and AAM2R effects were significantly greater in patients than in control subjects. *$p < 0.001$. **(B)** The percentages of autoantibody-positive patients for AAβ1AR and AAM2R and their combination are shown. Autoantibodies were significantly more frequent in patients with atrial fibrillation (AF) compared with patients with normal sinus rhythm (NSR). *$p < 0.001$.

with only 8 of 21 (38%) patients with sinus rhythm ($p < 0.001$). Likewise, 15 (88%) patients with AF were positive for AAM2R, compared with 4 (19%) patients with sinus rhythm ($p < 0.001$). Both autoantibodies coexisted in 14 (82%) patients with AF, compared with only 2 (10%) patients with sinus rhythm ($p < 0.001$).

Electrophysiologic effects of IgG. The electrophysiologic effects of IgG from 14 autoantibody-positive patients on canine pulmonary vein sleeves are summarized in Table 2. The IgG equivalent to a 1:100 serum dilution (0.15 mg/ml) reduced the resting membrane potential compared with pre-IgG values, increased the action potential amplitude, and decreased the action potential duration at 50% and 90% of repolarization (Fig. 2A). Pause-duration–dependent prolongation of the terminal action potential duration (action potential duration at 90% of repolarization) was enhanced after a 20-beat pacing train at 6 Hz for pause durations of 250, 500, 1,000, 2,000, and 4,000 ms, respectively, in the presence of IgG compared with control subjects ($p < 0.01$ for each pause duration). With rapid pacing followed by a prolonged pause, prolongation of the terminal phase of the action potential clearly assumes the form of an early afterdepolarization (Figs. 2B and 2C). Triggered firing with local autonomic nerve stimulation was observed in 50% and 79% of the pulmonary sleeve preparations before and after IgG, respectively (p = NS). The IgG decreased the voltage of the stimulus train needed to induce triggered firing, significantly moving the stimulus voltage-response curve to the left (EV_{50} = 70 ± 2 V vs. 96 ± 2 V after and before IgG, respectively, $p < 0.001$) (Figs. 2D and 2E). Early afterdepolarization formation and local autonomic nerve stimulation–induced triggered firing were blocked by atenolol (32 nmol/l). Hyperpolarization, action potential shortening, and local autonomic nerve stimulation-induced triggered firing were blocked by atropine (32 nmol/l).

Determinants of AF. Univariate analyses were performed for 14 variables, listed in Table 3. The copresence of AAβ1AR and AAM2R, old age, heart failure, and increased AAβ1AR (percent over baseline) and AAM2R effects (percent change over baseline) were significantly related to the presence of AF. Multivariate analysis showed the copresence of AAβ1AR and AAM2R was the strongest independent predictor of AF (OR: 33.61, 95% confidence interval [CI]: 1.17 to 964.11, p = 0.04). Older age also independently predicted the presence of AF (OR: 1.15, 95% CI: 1.02 to 1.31, p = 0.03) (Table 3).

To minimize the impact of age, we examined a (within 5 years) matched subgroup in our patient population. Ten patients with AF and 10 patients with sinus rhythm (mean age 54.3 ± 11.7 years vs. 53.0 ± 12.6 years, p = 0.81) could be compared. The copresence of AAβ1AR and AAM2R was significantly more prevalent in patients with AF (90% vs. 0%, p = 0.008).

AAβ1AR and AAM2R are distinct from TSHR antibodies. We examined the potential binding of AAβ1AR and AAM2R to TSHR expressed in CHO cells. Diluted TSHR

Table 2 **Electrophysiologic Effects of IgG (n = 14)**

	Pre-IgG	IgG (0.15 mg/ml)
Resting membrane potential (mV)	−74.6 ± 1.8	−76.9 ± 2.0*
Action potential amplitude (mV)	101.6 ± 7.6	105.7 ± 7.8*
Action potential duration at 50% of repolarization (ms)	44.3 ± 9.3	39.6 ± 7.0*
Action potential duration at 90% of repolarization (ms)	134.5 ± 17.8	118.0 ± 15.6*
Triggered firing (incidence)	50%	79%
Stim EV_{50} (V)	96 ± 7	72 ± 7†

*$p < 0.01$. †$p < 0.001$ versus pre-IgG.
Ig = immunoglobulin; Stim EV_{50} = stimulation voltage that results in 50% of the total incidence of triggered firing.

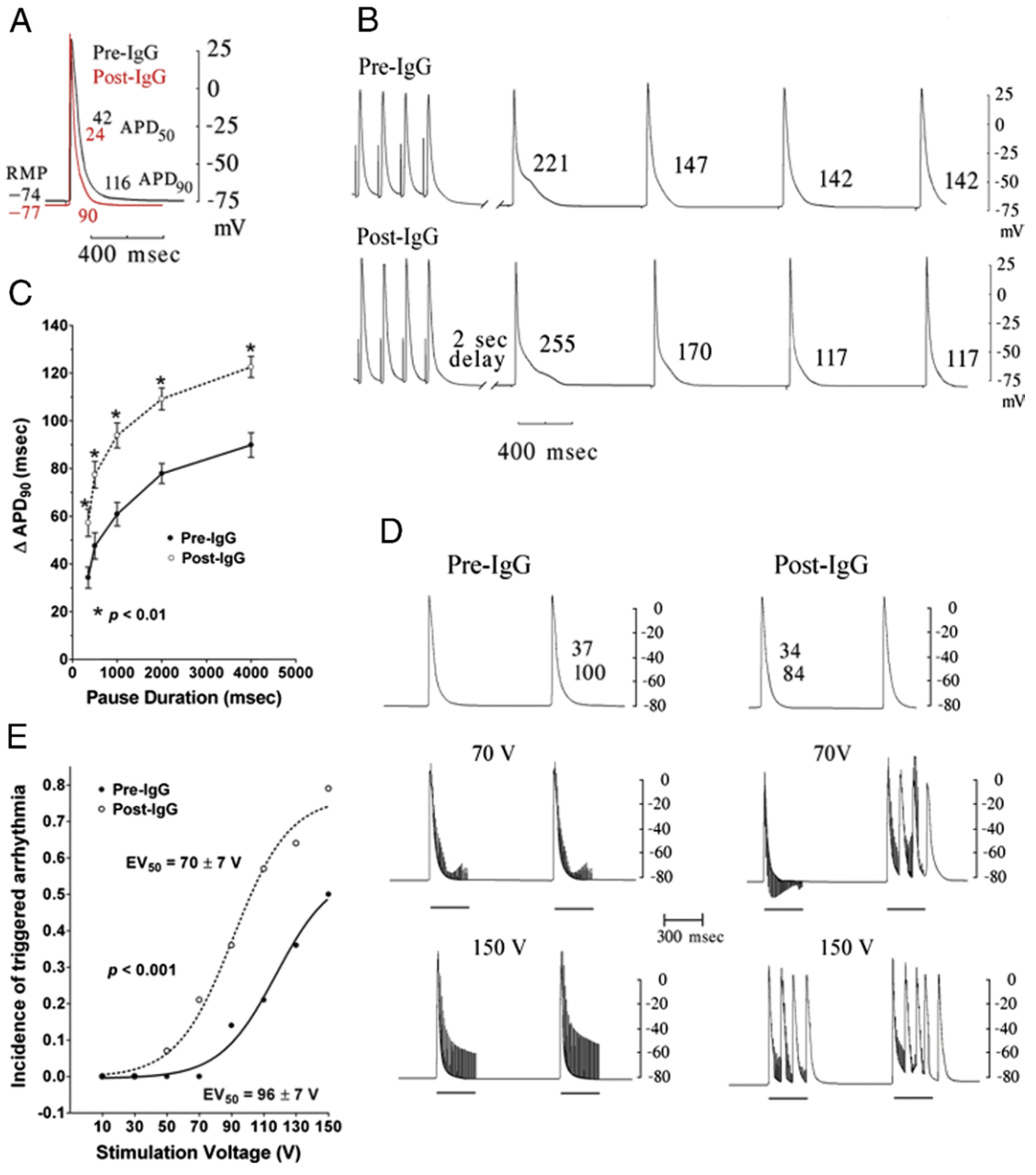

Figure 2 **Electrophysiologic Effects of IgG on Canine Pulmonary Vein Sleeves**

(A) Action potentials are shown before **(black)** and after **(red)** IgG administration demonstrating hyperpolarization, increased action potential amplitude, and decreased action potential duration. **(B)** Enhanced early afterdepolarization formation produced by tachycardia (6 Hz)-pause (2 sec) pacing is shown post-IgG in electrical recordings from pulmonary vein sleeves. Numbers represent action potential duration at 90% of repolarization. **(C)** Pause-duration–dependent prolongation of the terminal action potential duration (change in action potential duration at 90% of repolarization [ΔAPD_{90}]) with tachycardia (20-beat train at 6 Hz)-pause pacing is shown with IgG compared with control (n = 14). *p < 0.01 for each pause duration. **(D)** Electrical recordings from pulmonary vein sleeves during local autonomic nerve stimulation **(dark areas)** show enhanced triggered firing after IgG versus before IgG administration. The **numbers in the upper panel** represent the action potential duration at 50% and 90% of repolarization. **(E)** Stimulus response curves (local autonomic nerve stimulation) are shown before and after IgG administration, demonstrating enhancement of triggered firing by IgG in canine pulmonary vein sleeves (significant movement of the stimulus voltage-response curve to the left) (n = 14). APD_{50} = action potential duration at 50% of repolarization; RMP = resting membrane potential; other abbreviations as in Figure 1.

pre-adsorbed sera from 5 subjects; 4 with elevated AAβ1AR and AAM2R and 1 ELISA-positive but nonactive normal control subject were incubated with fresh CHO-TSHR cells in triplicate. These cells were labeled with anti-IgG antibodies and subjected to flow cytometry. Nonadsorbed sera from the same patients were used for control subjects. There was little binding and a <25% decrease in binding after adsorption in the nonactive normal control subject and

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Table 3 Univariate and Multivariate Logistic Regression Analyses for 14 Variables

Variable	Univariate			Multivariate		
	Odds Ratio	95% CI	p Value	Odds Ratio	95% CI	p Value
Copresence of AAβ1AR/AAM2R	44.33	6.51–301.90	<0.001*	33.61	1.17–964.11	0.04*
Age (yrs)	1.10	1.03–1.18	0.006*	1.15	1.02–1.31	0.03*
Male sex	1.19	0.33–4.29	1.00			
Hypertension	1.48	0.38–5.79	0.73			
Diabetes mellitus	1.75	0.43–7.18	0.49			
Coronary artery disease	2.01	0.30–13.86	0.64			
Congestive heart failure	3.60	0.90–14.37	0.09†	0.84	0.05–15.40	0.91
Ejection fraction (%)	1.00	0.96–1.04	0.92			
Left atrium diameter (mm)	1.09	0.98–1.21	0.13			
E/E′	0.73	0.32–1.69	0.49			
Serum thyrotropin concentration (mU/l)	1.21	0.01–118.42	0.94			
Serum free thyroxine concentration (ng/dl)	0.77	0.50–1.18	0.23			
AAβ1AR effect	1.13	1.04–1.21	0.002*	1.06	0.93–1.20	0.37
AAM2R effect	1.13	1.04–1.22	0.003*	1.07	0.94–1.22	0.31

*p < 0.05. †p < 0.10.
AAβ1AR = activating autoantibodies to the β1-adrenergic receptor; AAM2R = activating autoantibodies to the M2 muscarinic receptor; CI = confidence interval; E/E′ = ratio between the early diastolic velocity of mitral inflow (E) and that of mitral annulus (E′).

in the 2 non-Graves' subjects that were negative for TSHR antibodies. By contrast, the 2 subjects with concurrent TSHR antibodies and activating autonomic receptor antibodies had significantly higher baseline binding and a >50% decrease in TSHR binding after adsorption (Fig. 3). Serial dilutions of the adsorbed and nonadsorbed sera were examined by ELISA using β1AR and M2R. There was no significant loss in the adsorbed IgG reactivity to the autonomic receptor targets (data not shown).

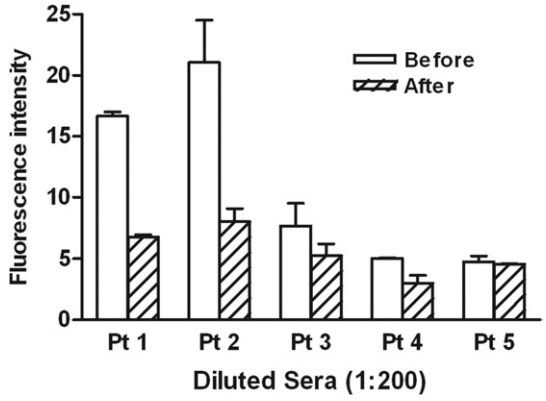

Figure 3 Binding of Patient Sera to CHO-TSHR Cells by Flow Cytometry

Fluorescence was measured after exposing these cells to 1:200 dilutions of purified IgG (before and after adsorption with TSHR) and fluorescein isothiocyanate-labeled anti-human IgG. A 50% decrease supports a significant loss of binding activity. Patients #1 and #2 had documented stimulating thyrotropin receptor antibodies and coexisting activating autoantibodies to AAβ1AR and AAM2R. Patients #3 and #4 were non-Graves' patients harboring AAβ1AR and AAM2R. Patient #5 was a control subject with nonactivating autoantibodies to β1AR and M2R by ELISA. There were significant adsorbable antibodies to the TSHR in the 2 Graves' patients. The other 3 had either no or small amounts of adsorbable activity. CHO-TSHR = Chinese hamster ovary cells expressing full-length thyrotropin receptor; ELISA = enzyme-linked immunosorbent assay; other abbreviations as inFigure 1.

Discussion

Graves' disease and autoantibodies. A significant percentage of patients with Graves' disease have activating autoantibodies against the β1AR and M2R. This increased frequency was observed mainly in patients with AF. Autoantibodies were present in some patients with sinus rhythm with a frequency greater than the 10% of a normal population reported in a previous study (22). This correlates with the fact that Graves' disease is an autoimmune disease (6,7). Thyroid-specific autoantibodies, such as thyroid peroxidase antibodies and TSHR antibodies, are present in 75% and 90% to 95% of patients with Graves' disease, respectively (6,23). The genetic, environmental, and endogenous factors responsible for the pathogenesis of Graves' disease increase the propensity of these patients to develop other autoantibodies (6).

Graves' hyperthyroidism and AF. In our study, traditional risk factors including hypertension, heart failure, increased left atrial diameter, and increased left ventricular filling pressures (as predicted by E/E′ ratio) did not identify AF risk in patients with Graves' disease. Stimulation of atrial M2Rs facilitates initiation and maintenance of AF (18), and AAM2R facilitates AF in patients with dilated cardiomyopathy (13). Our results likewise indicate that AAβ1AR and AAM2R facilitate AF formation in Graves' hyperthyroidism. Recent experimental findings describing rapid triggered firing from the canine pulmonary veins (16–19,24) provide important insights into a possible mechanism for these observed arrhythmogenic effects of AAM2R and AAβ1AR in patients with Graves' disease. Simultaneous activation of sympathetic and parasympathetic outflow from

ganglionated plexi located on the epicardial surface of the atrium (18,19), local stimulation of both parasympathetic and sympathetic nerve endings (17), and simultaneous administration of acetylcholine plus norepinephrine (or isoproterenol) (16) all initiate rapid triggered firing from canine pulmonary veins. The M2R activation (action potential shortening) and β1AR activation (enhancement of the calcium transient) are important and necessary components for such triggered firing. Herein, we provide evidence suggesting that both AAM2R and AAβ1AR exert sufficient electrophysiologic effects on pulmonary vein sleeve myocardium to facilitate triggered firing. Shortening of the action potential (AAM2R effect) and enhancement of tachycardia-pause early afterdepolarization formation (AAβ1AR effect) can generate an increased sodium–calcium exchange inward current and early afterdepolarization formation (24). In the concentrations used within the isolated pulmonary vein sleeve, the antibodies induced early afterdepolarizations, but were not sufficient alone to provoke triggering. However, the antibodies facilitated the generation of triggered firing elicited by local autonomic nerve stimulation. Although excess thyroid hormone per se can cause shortening of the action potential duration in atrial and pulmonary vein myocytes (4,5), the effects of AAβ1AR and AAM2R resulted from activation of their respective receptors because their effects could be blocked with the β-blocker atenolol and M2R blocker atropine, respectively. Elimination of the observed electrophysiologic effects with β-adrenergic and M2 muscarinic blockade suggests that it is unlikely that the TSHR autoantibodies directly caused these effects. Evidence from 4 subjects suggests that the autoantibody effects did not result from cross-reactivity of TSHR autoantibodies with the β1AR and M2R. These data from our ex vivo experiments are consistent with the concept that autoantibody activation of both β1AR and M2R facilitates initiation and maintenance of AF in patients and is responsible in part for the high incidence of AF in Graves' hyperthyroidism.

Age was an independent predictor of AF in our patient population, as in other studies (2,6). Autoantibody prevalence also increases with age in the normal population (22). It is likely that age, activating autoantibodies, and thyroid hormone act synergistically in this population.

Study limitations. It is possible that age differences in the AF and non-AF groups in this observational cross-sectional study might confound our data. Although not a case-control study, in an age-matched subgroup of our patient population the association between the copresence of AAβ1AR and AAM2R and AF remained highly statistically significant. We did not use long-term monitoring to identify patients in the non-AF group with unrecognized episodes of AF. However, the high rates of AF in patients with Graves' hyperthyroidism make the identification of a significant number of such episodes unlikely. The results of the multivariate analysis, although of interest, are limited by the relatively small number of observations included in the model and should be interpreted with caution. Finally, although other autoantibodies directed toward other receptors might exist, it is unlikely that they exert a significant electrophysiologic action, because the observed electrophysiologic effects were blocked completely with atenolol and atropine.

Conclusions

In patients with Graves' hyperthyroidism, the copresence of AAβ1AR and AAM2R facilitates autonomic-induced rapid triggered firing in pulmonary veins and is the strongest independent predictor of AF. These unique activating autoantibodies may play a role in the initiation and maintenance of AF in this patient population.

Reprint requests and correspondence: Dr. David C. Kem, Heart Rhythm Institute, TCH6E103, 1200 Everett Drive, Oklahoma City, Oklahoma 73104. E-mail: david-kem@ouhsc.edu.

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Key Words: activating autoantibodies ■ β-adrenergic receptors ■ M2 muscarinic receptor ■ atrial fibrillation ■ Graves' hyperthyroidism.