Autoimmune Hypertensive Syndrome

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Intravenous infusion of epinephrine or norepinephrine produces hypertension and symptoms similar to a pheochromocytoma.1,2 The sequelae are predictable on the basis of continuous infusion of agonists that activate β and α adrenergic receptors (ARs). These monoamine agonists, however, are not the only means by which β-ARs can be activated. Activating autoantibodies (AAs) directed toward the β/AR and/or the β-AR have been demonstrated in some patients with idiopathic dilated cardiomyopathy,3–5 Chagas’ disease,6,7 and other forms of cardiomyopathy.8,9 Several studies have demonstrated that these antibodies possess the ability to activate the intrinsic β/AR signal transduction system; however, these studies have focused only on the possible relationship of the AA/AR agonist effects on cardiomyopathy. Because β-agonist excess also produces changes in systemic blood pressure, one would expect the presence of AA/AR to produce a phenotype including some features observed in patients with an epinephrine-only secretory neuroendocrine tumor. Isolated studies have reported the concurrence of activating autoantibodies to the α/AR10,11 or to the angiotensin receptor (AT1)12,13 in a few patients with associated hypertension.

Herein, we present a hypertensive patient with no clinical evidence for a pheochromocytoma secreting excess catecholamine(s). However, in year 2000, there was conclusive evidence for anti-β/AR autoantibodies, which in vitro activate the β/AR signal transduction system independent of the intrinsic hormone-mediated system. Subsequent study in sera obtained in 2006 confirmed these data. These autoantibodies, as a result of their peptide sequence specificity, do not activate the β-adrenergic transduction system; as would norepinephrine or epinephrine at higher dosages. The evidence that these antibodies possess all of the β-activation potential of epinephrine is not unlike that expected with an epinephrine-only secreting pheochromocytoma but without a secretory tumor. The patient may represent the prototype of a new hypertensive syndrome that has an autoimmune basis.

Methods

Patient Presentation

Patient K113, aged 40, presented in year 2000 with poorly controlled type 1 diabetes mellitus requiring 30 to 50 IU of insulin per day. She was noted to have mild hypertension without blanching or flushing episodes, but she had postural hypotension. She was first diagnosed with type 1 diabetes mellitus at age 21 years and her diabetes had been poorly controlled on variable insulin regimens. Her heart catheterization demonstrated a globally dilated left ventricle with an ejection fraction of 30%. Diastolic dysfunction was observed with an end-diastolic pressure of 20 mm Hg. Coronary pathoanatomy showed minimal luminal irregularities with 1 plaque at ~40%, but no others were observed. The diagnosis she was assigned was “diabetic” cardiomyopathy complicated with hypertension. She was referred to the University of Oklahoma Endocrinology Section for consideration of an insulin pump. Initial studies revealed a hemoglobin A1c of 10.6, a blood pressure of 147/92 mm Hg while on 2 antihypertensive medications, and a 24-hour urinary protein >800 mg/d. Initial studies and sera were drawn, and a return visit was planned. In the interval, 1 month later, she suffered a myocardial infarction and had the documented onset of ventricular tachycardia leading to ventricular fibrillation and the sudden cardiac death syndrome. She was successfully resuscitated but suffered significant hypotension and resultant acute renal failure superimposed on her diabetic nephropathy.

She was subsequently placed on an insulin pump and achieved reasonable control of fasting and postprandial blood sugar excursions. Her total insulin requirements on this overall regimen dropped to 25 to 30 U/d, and occasional hypoglycemia was observed if she altered her meal timing. Her cardiac function was monitored by echocardiography, which provided evidence for a cardiomyopathy with a small region of the left posterior ventricle demonstrating impaired contractile function. Her estimated left ventricular ejection fraction was 28% in 2000. She was started on chronic renal hemodialysis but never recovered sufficient renal function to discontinue end-stage renal disease hemodialysis. Her physician placed her on the β-blocker carvedilol, clonidine, a calcium channel blocker, and a loop diuretic, and hemodialysis was initiated. She gradually improved, and her cardiac function gradually improved with an ejection fraction estimated at 35% 1 year later. Her medications varied over the next 5 years but always incorporated the β-blocker carvedilol and a low dosage of the converting enzyme inhibitor lisinopril. Her elevated blood pressure was reduced and remained in the 110/60- to 70-mm Hg range with a marked reduction of medications. An increase in postural hypotension with basal mildly elevated pulse was attributed to autonomic neuropathy. A sensori-neural deficiency was also noted. Later, markedly elevated AA/AR titers were detected in her original sera. She was brought back for follow-up studies under an established protocol approved by the institutional review board. Leukocytes also were obtained for follow-up studies and production of monoclonal antibodies.

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Because these elevated values of AAβAR (Figure 1) could cause a state of continual β-agonist activation, a limited evaluation to confirm the absence of a concomitant pheochromocytoma was performed. Urinary studies were unreliable because of the patient's marked anuria with concomitant hemodialysis. While at rest, a plasma epinephrine obtained from a cannula 30 minutes after insertion was 59 pg/mL (<84 pg/mL). The concomitant plasma norepinephrine was 171 pg/mL (<420 pg/mL). Analysis for serum chromogranin A was not possible because of concurrent renal failure. A computed tomography of the abdomen demonstrated 420 pg/mL). A limited evaluation to confirm the absence of a concomitant pheochromocytoma was performed. Urinary studies were unreliable because of the patient’s marked anuria with concomitant hemodialysis. While at rest, a plasma epinephrine obtained from a cannula 30 minutes after insertion was 59 pg/mL (<84 pg/mL). The concomitant plasma norepinephrine was 171 pg/mL (<420 pg/mL). Analysis for serum chromogranin A was not possible because of concurrent renal failure. A computed tomography of the abdomen demonstrated normal adrenal glands. Her cardiac status was reevaluated by optical density value of 0.10 at 405 nm in an ELISA plate reader (Dynex). Tests were in triplicate, and in each assay the titration was performed in triplicate assays and demonstrated almost no variation in optical density (∼0.1) from day-to-day assays.

Control Subjects
Twenty-five subjects were obtained who were age 18 to 80 years, male and female, all without evidence for preexisting hypertension, diabetes mellitus, or any cardiac illness. This study was approved by the University of Oklahoma Health Sciences Center Institutional Review Board. All of the subjects provided written informed consent.

ELISA
The ELISA was performed as described. Briefly, Immulon 4 (Dynatech Laboratories) microtiter plates were coated according to standard procedures at 4°C overnight with purified human cardiac myosin, human βAR, or β2AR expressed in S99 membranes (PerkinElmer) at 10 μg/mL in 0.1 mol/L of carbonate-bicarbonate coating buffer (pH 9.6). Plates were washed with PBS containing 0.05% Tween 20, blocked with 1% BSA in PBS for 1 hour at 37°C, and washed with PBS/Tween 20. To determine antibody titer, sera were diluted 1:100 in 1% BSA in PBS and thereafter diluted 2-fold. Sera were added to microtiter wells and incubated overnight at 4°C. Plates were then washed with PBS/Tween 20, and 50 μL of goat anti-human IgG secondary antibody (Sigma) conjugated with alkaline phosphatase at a 1:500 dilution was added and incubated at 37°C for 1 hour. Plates were finally washed with PBS/Tween 20, and 50 μL of substrate para-nitrophenyl-phosphate 104 (Sigma) in 0.1 mol/L of diethanolamine buffer (pH 9.8) was added. Optical density was measured at 405 nm in an ELISA plate reader (Dynex). Tests were in triplicate, and in each assay the titration was performed in duplicate. Titers were determined as the highest dilution with an optical density value of 0.10 at ∼60 minutes as compared with positive and negative standard sera. Controls included serum alone, secondary antibody conjugate alone, and 1% BSA alone. Reproducibility within each assay was monitored between duplicate and triplicate assays and demonstrated almost no variation in optical density (±0.1) from day-to-day assays.

Hybridoma Production
Peripheral blood was obtained from the patient. Mononuclear cells were separated from whole blood by Histopaque-1077 Hybri-Max (Sigma) and stimulated for 1 week with 10 μg/mL of streptococcal peptidoglycan-polysaccharide (Fisher Scientific) in Iscove’s Modified Dulbecco’s Medium containing 10% human AB serum. The cells were stimulated with pokeweed mitogen (1 μg/mL) in Iscove’s Modified Dulbecco’s Medium containing 10% human AB serum for an additional week. Cells were washed 3 times in Iscove’s Modified Dulbecco’s Medium without serum and fused with HMMA2.11TG/0 cells (human/mouse myeloma cell line, Dr Marshall Posner, Dana Farber Cancer Institute, Boston, MA) using polyethylene glycol-1000, as described previously. Hybridomas were selected by culture in hypoxanthine aminopterin thymidine medium and screened using GlcNAc conjugated to BSA as described. Cloning of hybridomas was achieved by limiting dilution and was performed 3 times. Established clones were maintained in Iscove’s Modified Dulbecco’s Medium containing 20% FBS.

Contractions Bioassays
Five- to 7-mm segments of free-running Purkinje strands from canine hearts were transferred to a 36°C perfusion chamber mounted on the stage of an inverted microscope (Olympus). The contraction was recorded using a video edge detector (Model VED-205, Crescent Electronics) at ×25 optical magnification, sampled at 120 Hz and recorded digitally. Fibers were stimulated with supermaximal constant current pulses of 4 ms at 1.0 Hz via extracellular platinum electrodes. The extracellular buffer solution (in mmol/L: NaCl 145, KCl 4.5, CaCl2 1.8, MgCl2 1, Na2HPO4 1, glucose 11, and HEPES/NaOH 15; pH 7.36) at 36°C was used. After achieving stable contractile responses, IgG from the patient, equivalent to a 1:100 serum dilution, was fed into the perfusion chamber at a rate of 5 mL/min. Contractions were continuously recorded before, at the onset, during steady effects of the test substance, and after the washout. After the washout, a second infusion of the same concentration of IgG plus propranolol (1 μmol/L) was performed to determine the degree of βAR activation attributable to the IgG. The βAR agonist isoproterenol (ISO; 10 nmol/L) was used as a positive control. IgG from normal subjects was used as negative controls. Contractility indices were recorded and analyzed using pClamp 9.2 (Axon Instruments) and Sigmasstat statistical software. An increase in contractility was considered significant when the P value was <0.05 (n=15 contraction cycles) using a multiple-measures ANOVA test. This technique is quite stable. The coefficient of variation at baseline (buffer only) was 4.4% and 4.6% after stimulation (values equivalent to ISO control; n=11 observations).

Automatisms Bioassays
These were performed in free-running Purkinje fiber preparations attached to a canine endocardial section in a perfusion chamber. This was perfused with a modified Tyrode’s solution at 36°C. Intracellular potentials were recorded from the central Purkinje strand using a glass microelectrode filled with KCl (3.0 mol/L). A bipolar electrogram was recorded from the distal free-running Purkinje strand, proximal to the endocardial insertion. Rates were determined after reaching equilibrium, generally within 1 to 2 minutes of exposure to the test substance. The average recorded rate was computed from 1-minute intervals. Rates were allowed to stabilize, and the test serum was then infused as described above. Serum infusion was followed by a 10- to 15-minute wash to allow a new baseline to be established. When the effect of a β-blocker was tested, it was first added to the perfusate to allow the new baseline to be established. Then serum was added into the buffer/β-blocker containing perfusate to determine whether the previously documented β-agonist activity was altered. Three separate measurements were made for each observation period, and the values represent the mean for each period. The coefficient of variation at baseline (buffer) was 2.4% and 2.6% after stimulation over an average of 20 consecutive beats.
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inhibition of antibody-induced PKA activity, the measured using a SignaTECT PKA assay system (Promega). For before homogenization. Protein kinase activity of H9c2 cells was centrifuged, and solubilized in 0.3 mL of protein extraction buffer ice-cold PBS. Cells were mechanically dislodged from flasks, of 15 mL of medium for 1 hour before reactions were stopped by (1:100 dilution) or IgG were incubated with cells in a final volume from the patient's serum using the Melon Gel IgG purification kit (Pierce Biotechnology). IgG was diluted to give a concentration in as described19 using serum from the patient and/or with IgG purified from the patient's serum using the Melon Gel IgG purification kit (Pierce Biotechnology). IgG was diluted to give a concentration in the bioassay equivalent to a 1:100 dilution of the patient's sera. Briefly, 1×10⁷ rat primary cardiac H9c2 cells (American Type Culture Collection) were plated in T75 culture flasks overnight. Sera (1:100 dilution) or IgG were incubated with cells in a final volume of 15 mL of medium for 1 hour before reactions were stopped by ice-cold PBS. Cells were mechanically dislodged from flasks, centrifuged, and solubilized in 0.3 mL of protein extraction buffer before homogenization. Protein kinase activity of H9c2 cells was measured using a SignaTECT PKA assay system (Promega). For inhibition of antibody-induced PKA activity, the β-blocker propranolol (10 μmol/L) was added. The βAR agonist ISO (10 μmol/L) was used to induce PKA activity in the H9c2 cardiac cells. Calculation of the units of PKA per microgram of protein was determined and then would wash out over a 5-minute period, indicating that the antibodies were not of such high affinity as to remain affixed for prolonged periods. This response was completely blocked when a β-blocker was added before the addition of the IgG. **P<0.01 vs control IgG.

### Protein Kinase Assays

Assays to determine protein kinase A (PKA) activity were performed as described19 using serum from the patient and/or with IgG purified from the patient's sera using the Melon Gel IgG purification kit (Pierce Biotechnology). IgG was diluted to give a concentration in the bioassay equivalent to a 1:100 dilution of the patient's sera. Briefly, 1×10⁷ rat primary cardiac H9c2 cells (American Type Culture Collection) were plated in T75 culture flasks overnight. Sera (1:100 dilution) or IgG were incubated with cells in a final volume of 15 mL of medium for 1 hour before reactions were stopped by ice-cold PBS. Cells were mechanically dislodged from flasks, centrifuged, and solubilized in 0.3 mL of protein extraction buffer before homogenization. Protein kinase activity of H9c2 cells was measured using a SignaTECT PKA assay system (Promega). For inhibition of antibody-induced PKA activity, the β-blocker propranolol (10 μmol/L) was added. The βAR agonist ISO (10 μmol/L) was used to induce PKA activity in the H9c2 cardiac cells. Calculation of the units of PKA per microgram of protein was determined and computed in the formula used in the assay kit purchased from Promega. The specific activity of the enzyme was determined in picomoles per liter per minute per microgram for each sample, and the results were presented as percentages above the basal level of PKA activity.

### Results

#### ELISA for AAβAR and Cardiac Myosin

Sera from the year 2000 demonstrated markedly elevated antibodies directed toward both β1 and β2 receptors as measured by ELISA (Figure 1). The ELISA titer for the AAβ2AR was 2-fold higher than AAβ1AR. These were 8- and 4-fold greater, respectively, than those observed in control subjects. There was a significant elevation of anticardiac myosin autoantibodies. Sera from 2006 demonstrated lower titers for each of the autoantibodies. These were just above the upper level of reference range (0 to 1600) for both βARs. The anticardiac myosin levels were within the reference range (0 to 800).

#### Activity Assays

Bioactivity was measured using a Purkinje fiber contractility assay. A dose response was examined using the year 2006 sera to establish parameters for subsequent activity assays. Serum dilutions of 1:400, 1:200, 1:100, and 1:50 were used (Figure 2, top). A small but significant increase in contractility was observed over baseline at the 1:400 dilution, and an increasing response was observed throughout the remaining dilutions. The remaining sera from 2000 and 2006, therefore, were used at a standard dilution of 1:100. Affinity-purified IgG also was used at a concentration of 1:100 of the estimated circulating IgG in the patient. A similar dose response was observed using ISO in concentrations spanning the concentration of 1:100 of the estimated circulating IgG in the patient. A similar dose response was observed using ISO in concentrations spanning the concentration range (0 to 800).
infusion of 10 nmol/L of ISO before application of the IgG, and a positive response was still observed with the IgG. Subsequent reperfusion with ISO demonstrated only modest evidence for desensitization to the IgG, and the positive ISO response was still retained.

The effect of the serum was also tested on the automaticity of the Purkinje fiber (Figure 4). A positive chronotropic response was observed that was relatively sustained over a 5-minute observation period. This accelerated response again was completely eliminated by previous treatment with the non-selective β-blocker nadolol (NAD).

Figure 4. The effect of the 2006 sera is shown on Purkinje fiber automaticity for the patient and a normal control subject. Each value represents the mean of 20 consecutive contractions and was measured once the preparation had stabilized. The data are expressed as the percentage above basal levels of the buffer control (dashed line; mean±SD; n=3). This effect was similar to the contractility data. There was no evidence for desensitization of the response over a 5-minute exposure period. This accelerated response again was completely eliminated by previous treatment with the non-selective β-blocker nadolol (NAD). *P<0.01 vs control sera.

The values are expressed as percentages above basal levels of PKA in medium control (dashed line) in the H9c2 heart cell line (mean±SD; n=3). There was no significant difference between the use of sera and affinity-purified IgG from the sera in the assay. The patient was not on a β-blocker when the first sample was obtained. There was no apparent effect of carvedilol on PKA activity in the diluted sera in 2006. This potential effect was eliminated in the purified IgG sample. The PKA activity was returned to baseline when propranolol (PROP) was added to the incubated cells. ISO was used as a positive control to activate PKA. *P<0.01 vs sera or IgG from a normal control subject.

realization that these antibodies have agonist capacity to illicit the pathophysiologic effects in vivo. These AAβARs target the βAR and activate downstream signal transduction, including PKA, when studied in vitro.19–22 AAβARs have been induced in experimental animal models, are associated with the onset of cardiomyopathy, and have been passively transferred from either a human source or from animal models to produce a cardiomyopathy.22–24 These characteristics fulfill the postulates of Witebsky et al25 for proof of an autoimmune cardiomyopathy. A most difficult corollary is to remove the antibody and thereby demonstrate improvement and/or cure of the disease. Although this has been attempted in a few poorly controlled studies,26,27 none are totally convincing, and a definitive study depends on more efficient means of suppressing or eliminating specific antibodies responsible for activation of the βAR. Although this patient was undergoing ultrafiltration hemodialysis, there were no data to support adding an experimental procedure, such as plasmapheresis, because her clinical status had stabilized.

Despite increasing evidence that AAβAR can mimic the presence of β-agonists, there has been little interest as to whether they are causative for hypertension. A review of several studies characterizing the presence or absence of AAβAR in patients with idiopathic dilated cardiomyopathy failed to provide individual data that might allow one to correlate whether systemic BP was maintained at a higher level in those with AAβAR compared with those without. In the animal passive transference studies, the tail BP was only recorded before and at the time when the cardiomyopathy had already developed. Because the associated decreased cardiac output frequently counterbalances any increase in systemic resistance in patients with primary cardiomyopathy, this may
not be a reliable sign pointing for or against the presence of AAβAR.

The patient herein described presents features compatible with an epinephrine-secreting pheochromocytoma. She had mild hypertension, diabetes mellitus (type 1 but with increased insulin resistance), lipid abnormalities, a cardiomyopathy, and susceptibility to a cardiac ventricular tachyarrhythmia associated with a subsequent myocardial infarct. These signs and symptoms were coexistent with markedly elevated titers of both AAβ1AR and AAβ2AR. These antibodies (IgG), at even a 1:100 or higher dilution of their expected circulating concentrations in sera, demonstrated significant agonist effects in 3 different activity assays. These include activation of PKA in heart cells in vitro, an increase in the cardiac contraction rate, and an increase in cardiac contractility. These effects were completely blocked by combined β1 and β2 blockade, and there was no evidence for residual α activation. This syndrome, therefore, differs slightly from that of a classic pheochromocytoma secreting only epinephrine, because the AAβARs are receptor selective, and there was no evidence for residual α1 activation. We did not have detailed hemodynamic studies other than the initial cardiac catheterization and measurements of cardiac function estimated from echocardiographic studies. Because even pheochromocytomas do not present with unique hemodynamic studies, we are unable to demonstrate any unique characteristics that might differentiate either type of adrenergic agonist presentation. Because the AAβARs do not change concentrations in a short frame of time and appear to not interact with the α1 receptor, one would not anticipate blanching. Although a type 2 diabetes mellitus is more characteristic of a pheochromocytoma, patients with autoimmune type 1 diabetes mellitus might be expected to develop autoimmune expression of AAβAR more frequently than the general population. This patient presented initially with increased insulin requirements before β-blocker therapy that could represent the expected metabolic effects of AAβAR.

Although there currently are no practical means less than repetitive selective or nonselective plasmapheresis for removing these AAβARs from the patient, long-term β-blockade was related to a marked improvement in the patient’s cardiovascular status. Although improved diabetic control after institution of an insulin pump probably contributed to this outcome, her overall glucose control still remained in a range that would be considered at risk. There was a significant attenuation of the titer of the AAβAR over a 6-year interval, and this may have been associated with her improved hemodynamic state and response to insulin.

**Perspectives**

Infusion of a β-agonist into an animal or human produces sequelae involving the cardiovascular and metabolic systems. However, the discovery of autoantibodies that activate βAR, even at serum dilutions of 1:100 or more, has not been easily accepted as contributing to the pathophysiology of hypertensive syndromes. The present study documents this probability. The pathophysiologic role of agonist-excess syndromes potentially caused by autoimmune activation expands our understanding of the range of signal transduction. Additional studies are needed to demonstrate the prevalence and significance of increased β-agonist activity associated with AAβAR in high-risk subjects. Many subjects within the hypertensive and/or diabetic population have sequelae that overlap with the present entity. It will be important to examine the prevalence and role that such activating antibodies have in their pathogenesis. It is possible that a subgroup of these patients have significant levels of AAβAR. There is reluctance by some clinicians to use β-blockers in patients with diabetes. However, the risks of decreased control and/or awareness of hyperglycemic episodes may not be as great as feared previously. The presence of significant titers of AAβAR with agonist effects would justify a high priority for a prospective study of β-blocker therapy and the use of other techniques to suppress and/or eliminate the AAβAR to demonstrate whether the full manifestation of the phenotype can be prevented.

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**Disclosures**

None.

**References**


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