Autoantibodies to β_2-Adrenergic Receptors: A Possible Cause of Adrenergic Hyporesponsiveness in Allergic Rhinitis and Asthma

Abstract. *Autoantibodies to β_2-adrenergic receptors have been identified in the serum of one patient with allergic rhinitis ("hay fever") and two patients with asthma. The antibodies precipitate solubilized dog lung β receptors in an indirect immunoprecipitation assay and inhibit the specific binding of iodine-125-labeled iodohydroxybenzylpindolol to membrane-associated receptors from dog lung, calf lung, and human placenta. Ligand binding to canine heart β_1 receptors is not affected by the antibodies.*

Autoantibodies to cell membrane receptors have been documented in a number of disease states in man. There is evidence for a pathogenic role for autoantibodies to the nicotinic acetylcholine receptor in myasthenia gravis (*1*), to the thyrotropin receptor in Graves' disease (*2*), and to the insulin receptor in certain types of insulin-resistant diabetes (*3*).

β-Adrenergic receptors exist on the surface of essentially all cells and play a significant role in the treatment of diseases such as asthma, heart disease, and hypertension. There has been little direct evidence, however, implicating β receptors in the etiology of disease. The "beta-blockade" theory of the pathogenesis of asthma (*4*) proposes that asthma is related to an imbalance in the autonomic control of airway diameter due to a decrease in β-adrenergic sensitivity in bronchial smooth muscle, mucous glands, and mucosal blood vessels. In this report we present evidence for the existence of autoantibodies to β-adrenergic receptors. Such antibodies may provide a primary mechanism for β-adrenergic "resistance" at the receptor level. The antibodies precipitate solubilized lung β receptors and block ligand binding to membrane-bound β_2 receptors.

Ten serums from patients attending an allergy clinic at the National Institutes of Health were screened for antibodies to human placental cell membranes, using ^{125}I-labeled protein A. The protein A assay has been utilized in screening serums for autoantibodies to insulin receptors (*5*). Three serums positive in the protein A assay, seven negative serums, and four control serums were coded and were then screened for their ability to precipitate solubilized canine lung β receptors (see below). Three of the 14 samples gave positive results in the β-receptor immunoprecipitation assay. When the results were decoded a direct correlation was observed between the samples that were positive in the protein A assay and those that precipitated solubilized β-adrenergic receptors. One serum was from a patient with allergic rhinitis and the other two were from patients with allergic asthma. Human placental membranes subsequently assayed were found to contain β receptors (30 fmole per milligram of membrane protein). Of the three positive samples, serum 10, from the patient with allergic rhinitis, had the highest titer in the β-receptor immunoprecipitation assay and was therefore chosen for further characterization.

To quantitate the effects of serum 10 on β-receptor precipitation, canine lung β receptors were labeled with [^{125}I]-iodohydroxybenzylpindolol (IHYP), a high-affinity β-receptor antagonist, and solubilized by a 15-minute treatment with 0.5 percent Triton X-100 at 30°C (*6*). The solubilized receptors were incubated with various dilutions of the serum and the receptor-antibody complexes were precipitated by addition of antiserum to human immunoglobulin G (IgG) (Fig. 1). Serum 10 precipitated lung β receptors in a dose-dependent fashion. At the lowest dilution tested (1:12), 30 percent of the total solubilized lung β receptors were specifically precipitated. Addition of the antiserum to human IgG in the final step of the assay was necessary for β-receptor precipitation. The IHYP alone was not precipitated by the serum or by addition of antiserum to human IgG. Because less receptor precipitation occurred at high detergent concentrations, the solubilized receptor complexes were first treated with Bio-Beads (*7*) to reduce the detergent concentration to approximately 0.05 percent.

The β-receptor autoantibody appeared to be directed at a determinant or determinants in or near the ligand binding site on the β receptor. Preincubation of

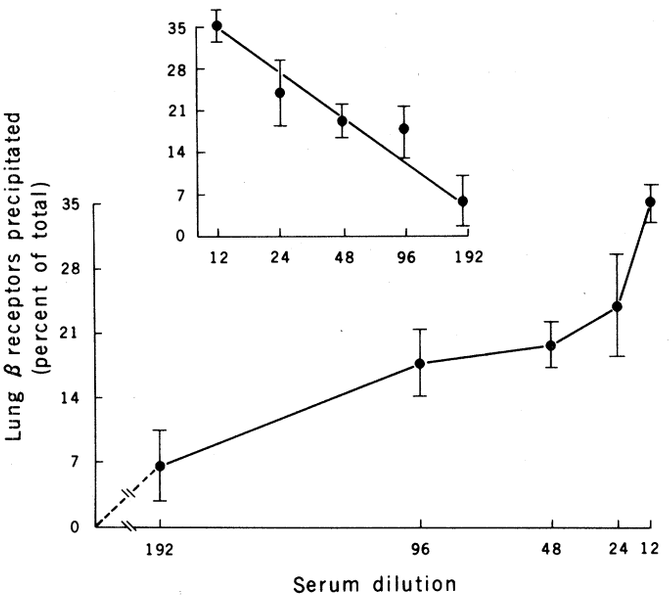

Fig. 1. Immunoprecipitation of solubilized canine lung β receptors by serum 10. Purified canine lung membranes were obtained by discontinuous sucrose density gradient centrifugation (*8*, *9*). Membranes were incubated with 250 p*M* IHYP in the presence and absence of 10 μM *l*-propranolol for 30 minutes at 30°C. The final concentration of membrane protein in the binding assay was 2 mg/ml. At the end of the incubation period, Triton X-100 in 20 m*M* sodium phosphate buffer (*p*H 7.4) was added to obtain a final detergent concentration of 0.5 percent. Membranes were incubated in Triton X-100 for a further 15 minutes at 30°C followed by centrifugation at 48,000*g* for 30 minutes (*16*). The supernatant containing the solubilized β receptors was then treated with Bio-Beads (Bio-Rad; 0.1 g per 0.5 ml) for 30 minutes at 4°C to reduce the detergent concentration approximately tenfold (*7*). Portions (100 μl) of the solubilized receptor-IHYP complex were added to 20-μl portions of serum 10, serially diluted in carrier control serum to the final dilutions indicated on the abscissa. The mixtures were incubated for 18 hours at 4°C, and receptor immunoprecipitation was then accomplished by addition of 100 μl of sheep antiserum to human IgG (2 mg/ml) for 6 hours at 4°C. The visible immune complexes were centrifuged at 12,000*g* for 5 minutes in a Beckman Microfuge, supernatant was aspirated, and precipitates were counted for radioactivity in a Beckman 4000 gamma counter. The same amount of protein was precipitated in all tubes. The total concentration of labeled receptor in an equivalent 100-μl sample was measured by a polyethylene glycol (PEG) precipitation assay at the start and the conclusion of the immunoprecipitation assay (*8*). Portions (100 μl) of the receptor-IHYP complex were added to 50 μl of gamma globulin (600 μg) at 4°C, followed by the addition of 1.2 ml of 15 percent PEG. Samples were mixed, incubated at 4°C for 20 minutes, and then centrifuged in a Beckman Microfuge. Supernatants were aspirated and the IHYP present in the precipitate was counted. Specific binding is defined as total IHYP bound minus IHYP bound in the presence of 10 μM *l*-propranolol (*8*). The amount of solubilized receptor-IHYP complex in a 100-μl sample was 1.4 fmole. All points represent duplicate determinations from two separate experiments. (Inset) Semilogarithmic plot of the immunoprecipitation data.

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membranes from canine lung, calf lung, and human placenta with various dilutions of the serum resulted in a concentration-dependent decrease in IHYP specific binding to β receptors compared to that in control serum (Fig. 2). Washing the membranes after exposure to the patient's serum had no effect on the ability of the serum to inhibit IHYP specific binding. In contrast, pretreatment of the serum with antiserum to human IgG before incubation with the membranes completely abolished its ability to inhibit IHYP specific binding. This confirms that the serum factor responsible for the inhibition of binding to the β receptor is an IgG molecule. It should be noted that serum 10 did not alter binding of ^{125}I-labeled insulin or ^{125}I-labeled epidermal growth factor to their respective membrane receptors in canine lung or human placenta (data not shown), suggesting that inhibition of binding was specific for the β receptor. Furthermore, serum 10, at dilutions of 1:50 to 1:500, had little or no effect on IHYP binding to dog heart β receptors (Fig. 2), consistent with the notion that heart and lung β receptors are distinct molecular entities (*8*, *9*) and that the autoantibody in this serum is highly specific for the β_2 receptor. These data indicate that the adrenergic ligand binding sites of β receptors within a given species may not be identical, but that the β-receptor subtype in a particular tissue may be phylogenetically conserved (*8*, *9*).

Serum dilutions that cause half-maximal inhibition of ligand binding to dog lung and calf lung membrane β receptors are 1:110 and 1:130, respectively, whereas the titer for immunoprecipitation of the dog lung β receptor is 1:60. The lower titer for precipitation could be due to the antibody being directed at a determinant in or near the adrenergic ligand binding site. It is also possible that the detergent used in lung β-receptor solubilization masks antigenic determinants, as demonstrated for immunoglobulin E receptors (*10*).

The specificity of the antibody for lung β_2 receptors enhances its potential significance as a mediator of allergic respiratory disease. The antibody titers for inhibition of ligand binding and β-receptor immunoprecipitation are comparable to those reported for insulin receptor antibodies in some insulin-resistant diabetics (*3*) and in New Zealand obese mice (*11*). These insulin receptor antibodies appear responsible for a syndrome of insulin-resistant diabetes, suggesting that at these low titers the anti-receptor antibodies can exert profound physiological effects.

Receptor blockade by β-receptor antibodies could upset the balance between β-receptor–induced relaxation of airway smooth muscle and the opposing influence of other mediators such as α-receptor agonists, histamine, prostaglandins, and acetylcholine (*4*, *12*, *13*). The β-receptor antibodies might also reduce receptor density on smooth muscle cells by accelerating the rate of β-receptor degradation, as with acetylcholine receptor antibodies in myasthenia gravis (*14*). Reduction of the β-receptor density of cultured human lung cells has been shown to affect the maximum cellular response to catecholamines (*15*), indicating that the density of β receptors can be a major determinant of physiological function. These data are consistent with the notion that autoantibodies to β_2 receptors may mediate β-adrenergic hyporesponsiveness in allergic rhinitis and asthma.

Our findings indicate the need for further screening of patients with allergic respiratory disease to establish the prevalence and pathogenetic role of autoantibodies to the β_2 receptor. In addition to their potential importance as mediators of disease at the molecular level, β-receptor autoantibodies are likely to be unique tools for the study of β-receptor structure and function and for the purification of β receptors.

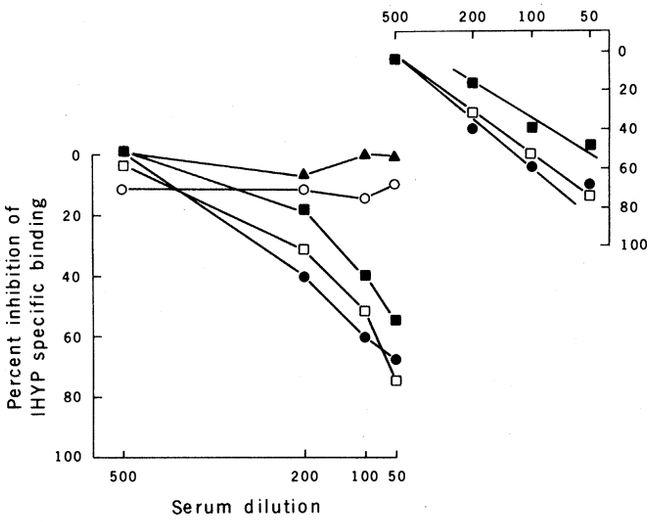

Fig. 2. Effect of serum 10 on IHYP binding to membrane-bound β-adrenergic receptors. Purified membranes (~ 100 μg of protein) from (○) canine heart, (□) canine lung, (●) calf lung, and (■) human placenta were preincubated with the indicated dilutions of serum 10 or control serum for 60 minutes at 30°C. Canine lung membranes were also preincubated under identical conditions with serum 10 that had been depleted of gamma globulin (▲) by immunoprecipitation with sheep antiserum to human gamma globulin. Dilutions of serum were made in assay buffer containing 50 m*M* Hepes and 4 m*M* $MgSO_4$, *p*H 8. The IHYP binding assay was performed (*8*) in polypropylene tubes in a final volume of 500 μl. The reaction was initiated by the addition of approximately 110 p*M* IHYP with or without 10 μ*M* *l*-propranolol, and the samples were incubated for 30 minutes at 30°C. The samples were then diluted with 1.25 ml of filtration buffer (20 m*M* potassium phosphate and 1 m*M* $MgSO_4$, *p*H 8) containing 0.1 m*M* *dl*-propranolol and immediately filtered through 2.4-cm Whatman GF/C glass fiber filters under low vacuum. The filters were washed with 25 ml of filtration buffer [without *dl*-propranolol] at 37°C and dried in a higher vacuum, and the radioactivity was counted in a Beckman 4000 gamma counter with 70 percent efficiency. Specific binding is defined as the difference in IHYP binding in the presence and absence of 10 μ*M* *l*-propranolol. Percent inhibition of IHYP specific binding was determined from the ratio of IHYP bound in the presence of serum 10 to that bound in the presence of control serum. Binding of IHYP to membrane β receptors in the presence of control serum at dilutions equal to or greater than 1:100 was not significantly different from that observed in the absence of serum. (Inset) Semilogarithmic plot of data for IHYP binding to purified membranes.

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16. The supernatant after centrifugation at 48,000*g* was considered to be the solubilized receptor fraction since no additional material sediments at 100,000*g* during a further 2-hour centrifugation. In addition, the detergent-extracted receptor-ligand complexes are retained by a 10,000-dalton Pellicon type PT membrane but not by Millipore HAMK 24 filters unless first precipitated by 15 percent polyethylene glycol, and are retained in a Sepharose 6B column with a calculated Stokes radius of 5.8 nm (*9*).
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Clonal Coexistence in *Daphnia pulex* (Leydig): Another Planktonic Paradox

Abstract. *Allozyme variation is common in populations of* Daphnia pulex *reproducing by obligate parthenogenesis. The genetic diversity within populations results from the coexistence of genetically different clones. Twenty-two clones were recognized in the eleven populations surveyed, of which as many as seven were found in a single habitat.*

A shallow well-mixed lake or pond is typically inhabited by a number of phytoplankton species, all competing for light and nutrients. Hutchinson (*1*) termed this the "paradox of the plankton," pointing out that such coexistence seems to violate the principle of competitive exclusion. Explanations of phytoplankton diversity have emphasized temporal and spatial heterogeneity in the environment (*1*, *2*) as well as the possibility that algal populations are limited by an array of nutrients (*3*). In contrast to the phytoplankton, early studies suggested that coexisting zooplankton species occupied distinct niches. Hutchinson (*4*), for example, pointed out that coexisting copepods generally differ widely in size and suggested that these size differences were correlated with the utilization of different resources. Recent work on cladocerans has clouded the issue. It is now recognized that many of the "species" described by classical systematists are species complexes (*5*). These morphologically similar species not only coexist but also use similar resources, despite the fact that populations are often resource-limited (*6*). The limits to coexistence are not clear. Genetic studies on *Daphnia magna* have shown that large numbers of clones often coexist (*7*). However, as this species is capable of sexual reproduction, it can be argued that clonal coexistence is short term, for new clones may enter the population from sexual eggs.

We now report the results of a study of allozyme variation in natural populations of *Daphnia pulex*, one of the most common zooplankters in pond habitats throughout Eurasia and North America. This species is normally thought to reproduce by cyclical parthenogenesis (*8*, *9*). Species of *Daphnia* reproducing in this fashion produce both parthenogenetic (*10*) and sexual eggs. The sexual eggs are enclosed within a protective structure known as an ephippium and must be fertilized if they are to develop. Some species such as *D. middendorffiana* and *D. cephalata* produce their ephippial eggs parthenogenetically —they are obligate parthenogens. There have been a few reports of populations of *D. pulex* reproducing in this fashion (*11*). We find that such asexual populations are not rare; all of our populations reproduce by obligate parthenogenesis.

We studied nine populations inhabiting ponds in urban or farmland habitats in southwestern Ontario, and two others in natural woodland pools. The habitats were filled with water only during the spring and early summer. The populations were sampled in the early spring shortly after their reestablishment from ephippial eggs.

Specimens for electrophoresis were stored alive at 5°C and used within 4 days of collection. The electrophoretic studies were carried out on single individuals. At least 48 individuals were analyzed from each population for the following enzymes: fumarase (FUM), glucose-6-phosphate dehydrogenase (G6PDH), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), tetrazolium oxidase (TO), xanthine dehydrogenase (XDH), amylase (AMY), and glutamate oxaloacetate transaminase (GOT) (*12*). Laboratory clones were established from at least 24 randomly chosen parthenogenetic females from each population (*13*). These clones were used to determine associations between genotypes at different loci.

Table 1. Genotypic frequencies at four polymorphic loci in populations of *Daphnia pulex* from southwestern Ontario. Frequencies were determined by electrophoresis of samples from single individuals taken directly from the populations. AMY 2 data are absent because of scoring difficulties.

Population	LDH			PGI				PGM						AMY1					
	N	SS	SF	*N*	SS	SF	FF	*N*	SS	SM	MM	MF	FF	*N*	SS	SM	MM	MF	FF
Cedar Springs	192	.005	.995	278	.126	.126	.748	189	.646		.339	.016		50		.02	.04	.94	
Charing Cross I	72		1.00	134		.746	.254	118	.263		.729			72				1.00	
Charing Cross II	48		1.00	202		.03	.97	174	.983		.017			44				1.00	
Cottam	309	.89	.11	216		.023	.977	201	.06		.264	.677		117				.12	.88
Kingsville	120		1.00	168			1.00	168	.006		.030	.964		141	.028	.035	.936		
Windsor I	222	.356	.644	95			1.00	287	.631		.369			71		.028		.761	.211
Windsor II	191		1.00	120			1.00	217	.350		.650			120		.658		.347	
Windsor III	186	.027	.973	96			1.00	216	.926		.014	.060		167			.042	.946	.012
Bloomfield	96		1.00	96			1.00	96			1.00			72				1.00	
Rondeau I	96	1.00		96		.26	.74	72	.028		.042	.333	.597	72				.014	.986
Rondeau II	72	1.00		48		.75	.25	47	.021	.021	.191	.745	.021	48				.104	.896

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