Retinal dysfunction in patients with chronic Chagas' disease is associated to anti-*Trypanosoma cruzi* antibodies that cross-react with rhodopsin

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SPECIFIC AIMS

To confirm the existence of a selective dysfunction of retinal rods in patients with chronic Chagas' heart disease (cChHD), we performed both clinical and electrophysiological studies (i.e., electroretinogram (ERG) and retino fluorescent angiography (RFA)). Serial biochemical experiments were designed to elucidate the molecular mechanism underlying the physiological abnormalities of the visual process. Cross reactivity between anti-*Trypanosoma cruzi* antibodies and human G-protein-coupled receptors (GPCRs) has been already described. As the photo pigment rhodopsin present in retinal rod cells is a member of this family of receptors, an interaction between antibodies against *T. cruzi* and rhodopsin is suspected. We aimed to investigate the role of specific monoclonal antibodies against this parasite and β-adrenergic receptors (β-AR) as well as of IgG fractions from cChHD patients in the interference of light signal transduction of the rod outer segments (ROS) membranes.

PRINCIPAL FINDINGS

1. Electrophysiological studies

We performed ERG to evaluate the functional and anatomical state of the retina in 45 patients with confirmed diagnosis of cChHD. The ERG is a recording of the eye's electrical response to a flash of light. The response typically consists of a negative-going a-wave, followed by a positive-going b-wave. The leading edge of the a-wave provides a direct measure of photoreceptor activity, while the b-wave provides a reflection of the action of glial and other cells and represents the voltage difference between a- and b-wave. The responses of cChHD patients evoked by all the white, red, or blue flash stimulations under the light-adapted state were normal. However, under the dark-adapted condition, using either dim blue or dim red flash stimulations, we observed a temporal dispersion of each response with a remarkable reduction of the b-wave amplitude when compared with controls (**Table 1**). Dim blue flash stimulation presented weak, delayed, or even nonexistent responses in the b-wave amplitude, in 36 of the 45 patients (80%) in one eye, and in 9 out of 45 patients (20%) in both eyes. Dim red flash stimulation evoked abnormal responses for b-wave latency and b-wave amplitude in 37 out of 45 patients (82.22%) in one eye, and in 19 of the 45 patients (42.22%) in both eyes. These abnormal responses tended to improve when dark adaptation time was prolonged up to 45 min suggesting a time-dependent adaptation rather than an all or none response-type deficit. RFA was performed in 33 of the patients that presented abnormal ERG. We observed weak to moderate defects, such as parafoveolar retinal pigment epithelium dispersion, general pallor and atrophy in the retinal epithelium of all these patients. These abnormalities were more evident at the posterior pole of the eye.

2. Biochemical studies: immunological assays

We examined the possible interaction between IgG fractions from cChHD patients with retinal dysfunction and rhodopsin from bovine ROS disk membranes by Western blot. IgGs from these patients recognized the 40 kDa protein band corresponding to bovine rhodopsin. We confirmed the 40 kDa protein band as rhodopsin by immunoblotting the membranes with the specific mAb E2 directed against the second extracellular loop of bovine opsin. This interaction was abolished by

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TABLE 1. *Registered data of a-wave and b-wave latencies and peak-to-peak b-wave amplitude between control and patients with chronic Chagas heart disease*[a]

STIMULUS	PATIENTS	ERG response			
		a-wave latency (ms)	b-wave latency (ms)	b-wave amplitude (microvolts)	recording ratio
Light-adapted condition-White flash	Control (n = 100 eyes)	17.33 ± .95	38.10 ± 2.20	17.12 ± 6.50	100/100
	CChHD (n = 90 eyes)	18.20 ± 2.18	39.34 ± 2.46	15.88 ± 6.60	90/90
Dark-adapted condition-Dim blue flash	Control (n = 100 eyes)	17.75 ± 2.00	38.22 ± 2.88	15.50 ± 5.35	100/100
	CChHD (n = 90 eyes)	19.44 ± 3.80	43.18 ± 4.48	*6.15 ± 3.88	63/90
Dark-adapted condition-Dim red flash	Control (n = 100 eyes)	18.44 ± 2.48	17.49 ± 3.20	14.45 ± 4.45	100/100
	CChHD (n = 90 eyes)	20.82 ± 3.12	*44.12 ± 5.18	*6.26 ± 3.12	55/90

[a] Values represent means of data obtained from the recorded eyes ± SD. * $P < 0.001$.

preincubation of mAb E2 in the presence of the specific E2p peptide. Subsequently, we probed the immunoblots with previously classified IgG fractions from cChHD patients. The results revealed that IgGs with anti-β1-AR cross reactivity strongly recognized rhodopsin, while those with selective anti-M2 cross reactivity did not. Preincubation of anti-β1-AR reactive human IgGs with the *T. cruzi* total homogenate strongly diminished rhodopsin detection. We could also observe the competitive effect of both R13 peptide, an antigenic peptide corresponding to the C-terminal epitope of *T. cruzi* ribosomal P2β protein (TcP2β), and E2p peptide, on the immunorecognition of rhodopsin by IgG fractions. We also probed immunoblots of ROS preparations with the mAb M16 directed against the second extracellular loop of the human β1-AR and, as expected, mAb M16 successfully recognized rhodopsin. We obtained the same result with the mAb 17.2, raised against R13 peptide. We demonstrated the specificity of each interaction by preincubation of mAbs M16 and 17.2 with peptides H26R and R13 respectively. This strongly diminished the interaction with rhodopsin. IgG fractions anti-M2 did not react with rhodopsin.

3. Biochemical studies: enzymatic assays

We measured the magnitude of light stimulation of rhodopsin-dependent guanosine 3′, 5′-cyclic monophosphate phosphodiesterase (cGMP-PDE) from bovine rod membranes in either the presence or the absence of IgGs from cChHD patients and monoclonal antibodies raised against *T. cruzi* and β-AR. mAb E2, a specific antibody against the second extracellular loop of rhodopsin, inhibited light stimulation of cGMP-PDE activity (**Fig. 1*A, B***); this inhibition was strongly reverted by the simultaneous incubation with E2p peptide. IgG fractions from cChHD patients that presented an exclusive β1-AR cross reactivity blocked rhodopsin-mediated light stimulation of cGMP-PDE activity, whereas IgG fractions with clear anti-M2 cross reactivity exerted no measurable effect. IgG fractions from healthy control individuals did not inhibit the enzymatic activity. The inhibitory effect of IgG fractions cross-reactive to β1-AR from cChHD patients was abolished by preincubation with *T. cruzi* lysate. The light-stimulated cGMP-PDE activity was also strongly inhibited by mAbs M16 and 17.2, but not by mAb anti-M2 receptor, and this inhibitory effect was clearly antagonized by H26R and R13 peptides respectively. IgG fractions from cChHD patients with abnormal ERG response inhibited light stimulation, but those belonging both to patients with normal ERG responses and to healthy individuals did not.

CONCLUSIONS AND SIGNIFICANCE

Chagas' disease is a major endemic disease in Central and South America, caused by the protozoan parasite *T. cruzi* and is characterized by an acute and a chronic phase. Tissular damage observed in affected organs even in the absence of the intracellular form of the parasite has led most of the researchers to propose an immunological mechanism to explain the pathogenesis of cChHD patients. It has been previously demonstrated that IgG antibodies present in the serum of these patients specifically bind to myocardium β-AR and muscarinic M2 receptor, thus suggesting that the targets of the autoimmune attack include some members of the large GPCRs family. An antigenic mimicry between *T. cruzi* and some autonomic receptors appears to be responsible for cross reactivity of antibodies from cChHD patients with normal GPCRs, thus affecting signal transduction. Rhodopsin, the dim light receptor pigment in the rods of the retina, like all GPCRs, activates a G-protein upon absorption of light. The biochemistry in the rod cell ultimately causes closing/opening of the cation conductance channels in the

plasma membrane through the activation of a cGMP-PDE. This results in hyperpolarization of the cell, activation of the synapses to the subsequent sets of cells

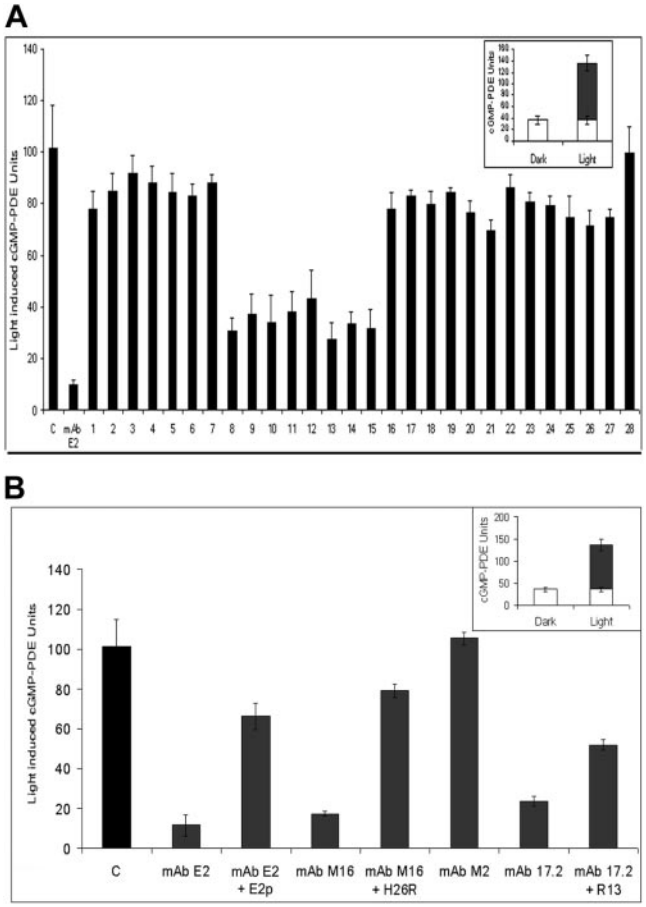

Figure 1. Monoclonal antibodies and IgG fractions from cChHD patients inhibit light induction of rhodopsin-dependent cGMP-PDE enzymatic activity from bovine ROS membranes. Enzymatic activity was performed on 1.5 μg of ROS membranes preparation (0.5 μM rhodopsin) in a final volume of 100 μL. *A)* IgG fractions from control patients scarcely inhibited cGMP-PDE activity (lanes 1–5). No inhibition of rhodopsin-dependent cGMP-PDE activity was produced by IgG reactive to M2 (lanes 6–7). IgG fractions from cChHD patients reactive to β1-AR significantly reduced the enzymatic activity (lanes 8–15), which was restored when preincubated with *T. cruzi* total antigen (lanes16–23). Reversion of inhibitory effect is also demonstrated with R13 (lanes 24, 25) and H26R (lanes 26, 27) peptides. No effect on enzyme activity light stimulation is produced by *T. cruzi* lysate (lane 28). *B)* Light-induced cGMP-PDE activity is strongly inhibited when preincubated with mAb E2. The magnitude of inhibition caused by mAb M16 was similar to that produced by the specific mAb E2. This inhibition was abolished when mAb E2 and M16 were preincubated with E2 and H26R peptides. Monoclonal antibody 17.2 showed similar inhibitor effect, which diminished by preincubation with R13 peptide. No inhibition was observed with mAb M2. *Inset)* Rhodopsin-dependent cGMP-PDE activity is expressed as the difference between light-stimulated (■) activity and basal activity (□) recorded under continue slow intensity red light (dark condition). Results shown here represent the mean of at least 3 experiments. Standard deviation was calculated with GraphPad Prism software, San Diego.

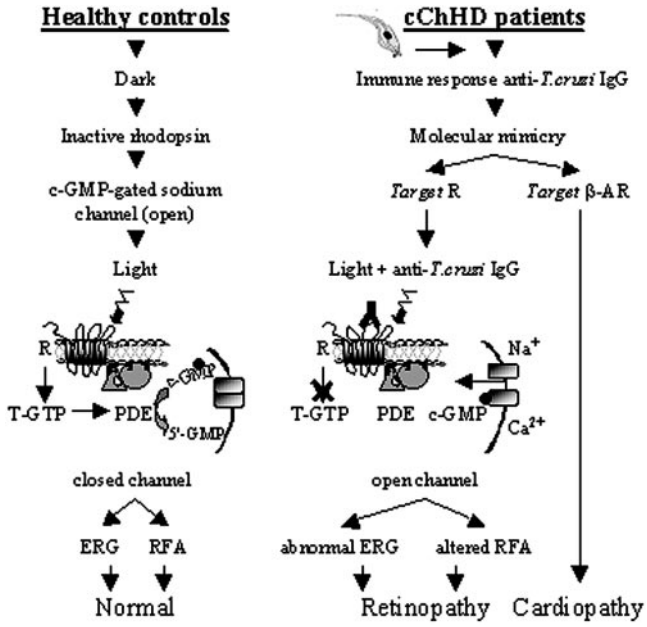

Figure 2. Schematic diagram depicting differential phototransduction events in the retin of healthy people or patients with cChHD. We propose a mechanism to explain the light signaling interference based both on the present findings and on those previously published about the molecular mimicry related to antibodies against *T. cruzi* and human receptor proteins.

in the retina, and eventually in a signal to the brain. Our results demonstrate a selective functional involvement of the rods in patients with cChHD and an in vitro molecular interaction between a specific type of IgG fractions from cChHD patients and rhodopsin. ERG evaluation of cChHD patients demonstrated a dissociated clinical retinal involvement with selective dysfunction of the rods in 80% of these patients. Defects in the parafoveolar peripheral retinal pigment epithelium described previously were confirmed in some of our patients, and correlate with the anatomical distribution of the rods. The inhibition of light-induced cGMP-PDE activity measured in the presence of IgG with affinity to β1-AR strongly suggests an interaction between these antibodies and rhodopsin. Attenuation of this functional inhibition by *T. cruzi* lysate as well as by R13 peptide would confirm the specificity of this antibody-mediated photoreceptor blockade. On average, the magnitude of interference on light signaling by IgG fractions from cChHD patients correlates with the magnitude of abnormalities of electro-ophtalmological records. In this report we describe a novel retinopathy consisting of selective dysfunction of light signal transduction in rod membranes from patients with cChHD. The results of the electrophysiological, biochemical, and immunological assays that we presented here strongly suggest a molecular interaction between a specific type of anti-*T. cruzi* antibodies and rhodopsin, an interaction that could be the cause of the selective functional involvement of the rods. FJ