Passive Administration of Antibody Against Retinal S-Antigen Induces Electroretinographic Supernormality

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Electroretinographic supernormality, affecting both the a- and b-waves of the electroretinogram (ERG), occurs consistently before the onset of experimental autoimmune uveoretinitis (EAU) in rabbits and rats. To investigate the possible role of antibody to S-antigen (S-ab) in this phenomenon, affinity-purified polyclonal rat S-ab was injected intravenously into normal rats and administered to isolated rat eyecup preparations by bolus perfusion. In both situations, ERG supernormality was observed. The effect in vivo peaked 90 min after injection, and ERG changes in vitro were observed within 15 sec. The ERG response in vivo and in vitro was dose dependent and was abolished in vivo by pretreatment with cyproheptadine (a serotonin antagonist). The ERG was not affected in either system by a control rat antibody (antiovalbumin) or by murine monoclonal or rabbit polyclonal antibodies to S-antigen. The induction of ERG supernormality in vivo and in vitro by homologous S-ab indicates the operation of species-specific mechanisms both involving and bypassing the blood–retinal barrier and highlights a significant role for humoral autoimmunity in the pathogenesis of S-antigen-induced EAU in the rat. Invest Ophthalmol Vis Sci 33:30-35,1992

Experimental autoimmune uveoretinitis (EAU) may be produced in several animal species by inoculating them with a retina-specific protein, retinal S-antigen. It currently is studied extensively as a model of certain forms of uveoretinitis in humans. Electrophysiologic monitoring of EAU showed an abnormal enhancement of the a- and b-waves in electroretinography (ERG) before any clinical or histologic signs of disease. In Lister rats, we reported that ERG supernormality characteristically occurred during week 3 after sensitization with S-antigen at a time when serum titers of antibody against this antigen had reached their peak. S-antigen is known to be important in the downregulation of phototransduction by binding to phosphorylated rhodopsin early in the amplified cascade. We therefore investigated whether antibody to S-antigen (S-ab) might induce ERG supernormality by inactivating endogenous S-antigen (thus allowing an uninhibited response to light in the photoreceptor) after administering affinity-purified S-ab to unsensitized animals.

We also considered whether this phenomenon might be caused by the breakdown of the outer blood–retinal barrier (BRB) and thus by changes in the extracellular environment of the photoreceptor. It was suggested that mechanisms of immediate hypersensitivity might play a role in EAU through antibody-mediated liberation of vasoactive amines from choroidal mast cells, leading to focal dysfunction of the BRB that others postulated might precede lymphocytic infiltration in the retina. Furthermore, recent electrophysiologic investigations of patients with early uveoretinitis also highlighted changes in this area. Because the main mast cell-derived amine in the rat is serotonin, we studied the effect of pretreatment with cyproheptadine, a serotonin antagonist, on the ERG responses of rats injected with S-ab. We further report ERG changes that occurred after perfusing an isolated rat eye cup preparation with S-ab, a situation where the BRB effectively was removed. A preliminary report of some of our in vivo work was published previously.

Materials and Methods

Adult black hooded Lister rats (Bantin and Kingman, Hull, UK) were used for ERG throughout, and rats of the same strain and rabbits were used for raising polyclonal antibodies. Treatment of animals was in compliance with the ARVO Resolution on the Use of Animals in Research.
Immunologic Reagents

Retinal S-antigen was prepared from bovine retinal extracts by size fractionation and hydrophobic adsorption chromatography. Rat polyclonal antisera against ovalbumin and bovine S-antigen were prepared by dissolving the relevant antigen in phosphate-buffered saline (PBS) and emulsifying it 1:1 in enriched Freund's complete adjuvant (FCA) containing 3 mg/ml of heat-killed Mycobacterium tuberculosis (MAFF, Weybridge, England). Rats received single foot-pad injections of 50 μg of either S-antigen or ovalbumin. Blood was obtained, and the sera were separated 4 weeks after the primary injection. Rabbit polyclonal antiserum against bovine S-antigen was prepared by injecting rabbits with 200 μg of S-antigen in FCA subcutaneously into multiple sites on the back followed by booster injections 3 weeks later of the same amount of antigen in incomplete Freund's adjuvant. Sera were obtained 2 weeks after booster injections. Mouse monoclonal antibodies against bovine S-antigen were raised in our laboratory and used as controls for species specificity.

Immunoglobulins were precipitated from pooled sera with ammonium sulfate at a final concentration of 40%. They were redissolved in PBS, dialyzed, and applied to a column of cyanogen bromide-activated Sepharose 4B (Pharmacia Biosystems Ltd, Milton Keynes, UK) onto which either S-antigen or ovalbumin had been coupled according to the manufacturer's instructions. The immunoglobulin solution was passed through the column at a flow rate of 12 ml/hr, and the column was washed with PBS until the protein content of the eluate fell below 0.05 mg/ml, as measured by optical density measurement at 280 nm (Beckman spectrophotometer; Beckman Instruments (UK) Ltd, High Wycombe, UK). Relevant antibodies were dissociated from the column by elution with 50 mM diethylamine, pH 11.5, and the eluate was neutralized immediately with solid glycine. The samples were assessed for protein by dye binding and were extensively dialyzed against PBS and stored at -80°C. The purity of the antibody was assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (Fig. 1). Its specificity was confirmed by solid-phase radioimmunoassay using S-antigen as the substrate.

ERG Recording

The ERG apparatus and protocols used in this study were described elsewhere. Briefly, bilateral full-field corneal ERGs were amplified (band width, 0.1–1000 Hz) and recorded conventionally using silver electrodes embedded in Perspex contact lenses on dark-adapted (≥1 hr) anesthetized (urethane, 1.5 g/kg) animals (body weight, 300–350 g). The ERGs were evoked by intermittent (0.5 Hz) xenon flashes (intensity, 40,000 cd/m²), and the animal's electrocardiogram and rectal temperature were monitored continuously.

Affinity-purified antibody was injected into a femoral vein cannula after the ERG amplitude reached steady state (30 min after the initial flash), and ERGs were monitored continuously for up to 5 hr postinjection. Eight animals received 50 μg of S-ab; 12, 250 μg; and 5, 500 μg. The a- and b-wave amplitudes and implicit times were measured conventionally. The positive b-wave amplitude of the ERG was expressed as a percentage of the preinjection level. Control, weight-matched animals had their ERGs recorded on the same day. The controls received mouse monoclonal antibody to retinal S-antigen antibody (three rats), rabbit polyclonal antibody to retinal S-antigen (five rats), and rat polyclonal antibody to ovalbumin (seven rats).

Cyproheptadine-treated rats: In this experiment, three rats were pretreated with cyproheptadine hydrochloride 10 mg/kg/day in PBS intraperitoneally for 4 days before ERG recordings, and three control rats received the vehicle (PBS) similarly. Both control and experimental animals were injected with 250 μg of affinity-purified rat S-ab under the recording conditions described. The animals were killed at the end of the experiment by an overdose of anesthetic, and their eyes were
removed and fixed in 4% glutaraldehyde followed by 10% formaldehyde for histologic studies. In vitro recording: The eyes were removed from anesthetized dark-adapted (>1 hr) rats and hemi-sected. The posterior chambers were mounted in a tissue bath designed for eyecup recording under dark-adapting conditions (Safelight; Kodak, Rochester, NY). The eyecup was everted over a dome in a Perspex well and continuously superfused with a mammalian Kreb’s solution (Fig. 2) maintained at 37 ± 0.5°C. The ERGs were recorded using silver–silver electrode pellets (Clarke Electromedical, Reading, UK), the indifferent electrode being mounted in the dome over which the eyecup was everted and the active electrode positioned on the vitreous side in the bathing solution. A combined amplifier (band width, 0.1–100 Hz) oscilloscope thermal recorder (Medelec MS92; Medelec Ltd, Woking, UK) was used to record ERGs. These were evoked using a xenon flash gun mounted on a trinocular eyepiece of a dissecting microscope positioned over the eyecup at a frequency of 0.01 Hz (28,000 cd/m²).

Control ERGs were recorded for 60 min, and then S-ab solutions were injected into the superfusate line (1 ml in 15 sec; flow rate, 10 ml/min) proximal to the heat exchanger to reach the preparation in less than 15 sec. Subsequently ERGs were recorded at 1-min intervals for 10 min and 5- to 10-min intervals thereafter. Initially ERGs were recorded from five control eyecups to show how the ERG behaved over time without experimental interference (Fig. 2). The viable life of the eyecup preparation was approximately 4 hr, enabling several experiments to be done on one preparation. Thus, alternate perfusions with antibody or vehicle were done every 15 min for five cycles, the ERG values having returned to those of the control at the end of each cycle. The results were expressed as the percentage of the maximum b-wave amplitude achieved after perfusion compared with the amplitude before perfusion.

Results

In Vivo Studies

Injection of affinity-purified rat S-ab caused a dose-dependent rise in the amplitude of both the a- and b-wave of the ERG with no change in the b–a ratio (Fig. 3). This effect was seen initially at 15 min and subsequently increased to reach a maximum at 90 min postinjection. In some animals, the rise continued for up to 5 hr, the amplitudes in these animals reaching tenfold their resting values. No change was found in the implicit times of either the a- or b-wave. In selected animals, we also observed a reduction in the ERG flicker-fusion frequency in tandem with the rise in amplitude (data not shown). In 90% of animals, the amplitude of the waves reached a maximum at 90 min and then began to decrease after 2 hr. The ERG effects in animals that received 500 ng of S-ab were similar to those of animals that received 250 ng (data not shown). Control animals, receiving either a rat antibody against an irrelevant protein (ovalbumin), a mouse monoclonal S-ab, or a rabbit S-ab, showed no change in any ERG parameter throughout the course of the experiment. No marked change was seen in electrocardiography or rectal temperature in any animal during these experiments.

Cyproheptadine Study

The results of pretreating animals with intraperitoneal cyproheptadine are shown in Figure 4. No pretreated animal showed any change in its ERG pattern over 2 hr; untreated animals showed the characteristic increase in amplitude expected for the dose of antibody (250 μg).

In Vitro Study

Figure 2 shows the normalized amplitude of the ERG b-wave (mean ± standard error of the mean for five eyecups) measured every 5–20 min without experimental manipulation. There were variable responses in the first 60 min before the ERGs stabilized caused by experimental interference; this period was not used for the study. The results of the eyecup experiments are summarized in Figure 5. The rise in the ERG amplitude occurred quickly (usually within 1 min) and
reached its peak within 10 min. In some experiments, the rise was sustained for up to 60 min. The maximum mean rise was 140% in vitro compared with 380% in vivo. Both the a- and b-waves were increased with no change to the b-a ratio. There was no change in the implicit time. None of the control antibodies or equivalent volumes of vehicle caused any deviation of the ERG parameters, and where these were injected, the ERG continued to decline as expected (Fig. 2).

Histologic Studies

Hematoxylin and eosin-stained sections from experimental and control animals were examined by light microscopy for abnormalities at the level of retinal
pigment epithelium and photoreceptor and also for evidence of cellular infiltration; none were found.

Discussion

Our study showed that administration of affinity-purified rat S-ab produced a dose-dependent enhancement of the ERG both when injected into normal rats and when perfused onto freshly isolated rat eyecup preparations. Because ERG enhancement occurred in both a- and b-waves, without affecting the b-a ratio, we believed that b-wave changes followed those of the a-wave and that the ERG effect was generated at the level of the photoreceptor cells, which contain S-antigen. The finding that ERG enhancement occurred in vivo and in vitro suggests that it was a direct consequence of immunoglobulin binding specifically to its target protein, whether or not S-ab crossed the BRB.

Available evidence supported two different mechanisms of action for these effects. First, injected S-ab might increase the permeability of the BRB, without actually crossing it, thus altering indirectly the extracellular biochemical environment of the photoreceptor layer. Although there is little evidence in the literature addressing this problem in vivo, studies in vitro show that reducing extracellular calcium will lead to an increase in a wave amplitude. One pathway for this could involve degranulation and release of vasoactive amines from mast cells in the choroid where S-antigen or its immunoreactive fragments are known to be available to specific antibody. On this basis, mast cell-derived vasoactive amines (mainly serotonin in the rat) would increase BRB permeability and thus lead to altered photoreceptor reactivity. This view would agree with evidence that degradation of mast cells occurs early in EAU before the onset of histopathologic changes in the retina. In addition, in our experiments, only the homologous (ie, rat) S-ab produced these ERG effects. Rat mast cells have receptors for Fc regions of homologous immunoglobulin E/IgG2a, and these isotypes might be represented among our rat polyclonal S-ab. Furthermore, the ERG effects were blocked in vivo by prior administration of cyproheptadine, a serotonin antagonist. This drug selectively blocks the action of serotonin on susceptible cells and can abrogate forms of experimental allergic encephalomyelitis that may share similar pathogenetic mechanisms with EAU. Thus, a sequence of localized mast cell activation, serotonin release, and BRB disturbance might be implicated in the ERG changes after passive administration of S-ab in vivo.

A second mechanism could involve passage of antibody across the BRB, interaction with the photoreceptor cell, and binding to photoreceptor S-antigen. An immediate effect of this could be to render the protein unavailable for terminating phototransduction. Thus, for a given amount of light energy, there would be overactivation of transducin and cyclic guanosine monophosphate phosphodiesterase, leading to an excessive electrical response. This response would be expected to increase further as more S-antigen is inactivated and to decrease as S-antigen either dissociates from antibody or is resynthesized in the photoreceptor cell. This mechanism requires that the rat S-ab can penetrate the photoreceptor cell membrane to be able to interact with cytosolic S-antigen. We suggest that the surface receptor for 11 cis-retinal that presumably transports this molecule into the photoreceptor cell could be involved in internalizing the rat antibody. The repertoire of rat polyclonal S-ab may include antibody directed to the epitope that normally recognizes activated rhodopsin and thus mimics the rhodopsin parent molecule (eg, 11 cis-retinal). After internalization, it may perturb photoreceptor cell physiology.

Our experiments show that both antibody-mediated mechanisms could be operating together in EAU. The in vivo model implicates both BRB breakdown and intracellular inactivation of S-antigen. The in vitro model suggests that inactivation of cytosolic S-antigen, occurring rapidly, may be followed by resynthesis of S-antigen or by dissociation of antibody, thus allowing the ERG to return gradually to normal. Whether either or both mechanisms underlie the ERG effects produced by S-ab, our study highlights a possible role for antireceptor autoimmunity in EAU. In particular, our findings question whether some antiretinal antibodies in EAU and in autoimmune human uveoretinitis can interact with cell-surface receptors of molecules involved in photoreceptor function. The unequivocal demonstration of autoantibodies to cell-surface receptors in EAU would help identify antiretinal autoimmunity mechanisms with those of autoimmune endocrinopathies.

Key words: electroretinogram, S-antigen, antibody, supernormality, rats

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References