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LABORATORY INVESTIGATION

Time-Dependent Course of Electroretinograms in the Spontaneous Diabetic Goto-Kakizaki Rat

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Abstract

Purpose: This study evaluates the relevance to human retinopathy of electroretinograms (ERGs) from the spontaneously diabetic Goto-Kakizaki (GK) rat.

Methods: Starting from 4 weeks of age, we obtained ERGs every 4 weeks from six GK rats and seven Wistar (control) rats, and from two GK and two Wistar rats at 14 days of age. The a-wave, b-wave, and oscillatory potentials (OPs) were recorded after stimulation with a single bright flash. We compared the amplitudes and implicit times and measured a-wave latencies to evaluate photoreceptor function.

Results: The amplitudes of the a-wave, b-wave, and OPs (O1 and O2) of the GK rats were reduced between 4 and 48 weeks of age. The time-dependent courses of change in a-wave, b-wave, and O2 amplitude did not differ between the two groups. The a-wave latencies in GK rats were significantly prolonged, but not the implicit times of OPs. At 14 days of age, the a-wave amplitudes were significantly smaller in GK than in Wistar rats.

Conclusion: Functional abnormalities of photoreceptors might be induced by inheritable degeneration at an early age in the GK rat. Although hyperglycemia would cause retinal hypoxia, it would not be severe enough to disturb the generation of OPs. **Jpn J Ophthalmol** 2006;50:211–216 © Japanese Ophthalmological Society 2006

Key Words: electroretinogram, Goto-Kakizaki rat, spontaneously diabetic rat

Introduction

Because diabetic retinopathy (DR) leads to blindness in diabetic patients, the pathogenesis of this condition should be understood in detail. Most studies have used animal models to generate histological and pharmacological information, and those using rats have revealed much valuable information.[1,2] Diabetes can be generated in rats using several strategies. For example, induction with drugs such as streptozotocin (STZ) is quite popular,[3] as it specifically injures β-cells in the pancreatic islets and thus decreases insulin release.[4] However, rats with STZ-induced diabetes rapidly develop cataracts,[5] and therefore retinal changes cannot be accurately evaluated. Furthermore, the effect of the drug itself cannot be ignored. However, these conditions can be resolved in spontaneous models. The Goto-Kakizaki (GK) rat is a model of spontaneous type II (non-insulin-dependent) diabetes mellitus without obesity, which was developed by repeated selective breeding of normal Wistar rats using glucose intolerance as the selective index.[6,7] Unlike drug-induced models of diabetes, the diabetic state is moderate, the plasma insulin response to glucose is impaired,[7,8] and almost all GK rats appear hyperglycemic. Because of the moderate diabetic state, cataracts do not develop rapidly. The GK rat is therefore considered a suitable animal model of human type II diabetes mellitus.

Electroretinogram (ERG) abnormalities among diabetic patients comprise a reduced b-wave amplitude and reduced or absent oscillatory potentials (OPs).[9] Before the onset of retinopathy and without visible changes in the retina, OP

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Jpn J Ophthalmol
Vol 50: 211–216, 2006

amplitudes become reduced.[10] Thus, OP is considered a sensitive indicator of DR[9,10,11] and should be examined in diabetic patients. Retinal changes are difficult to detect clinically at the onset of a glucose tolerance abnormality, because ophthalmoscopically visible alterations at that time are undetectable. Therefore, the mechanisms and implications of OP abnormalities must be determined in diabetic rats. However, because ERGs from GK rats have not been described, we evaluated the time-dependent changes in the components of ERGs from this rat model.

Materials and Methods

Animals

Male Wistar (n = 9) and GK (n = 8) rats from Pharmacological Research Laboratories (Japan SLC, Hamamatsu, Japan) were handled according to the principles of the ARVO statement for the Use of Animals in Ophthalmic and Vision Research. The animals were maintained at 22 ± 2°C, relative humidity, 55 ± 10% and a light/dark cycle of 14/10 h (light period, 8.00–22.00 h). A commercial diet (NMF; Oriental Yeast, Tokyo, Japan) and tap water were provided ad libitum.

Time Courses of ERG Recording

The blood glucose levels of GK rats (n = 6) were higher than those of age-matched Wistar rats (n = 7) from the age of 4 weeks. Therefore, we recorded ERGs and measured blood glucose levels every 4 weeks from that point using a portable system (Xtra; Abbott Laboratories, Abbott Park, IL, USA).

Group Aged 14 Days

Glucose intolerance becomes obvious in the GK rat around 2 weeks after birth.[7] Thus, we investigated whether hyperglycemia affects photoreceptor function in the GK rat by recording ERGs from both GK (n = 2) and Wistar (n = 2) rats at 14 days of age.

ERG Recordings

Surgical incision of the eyelids was required in the 14-day-old rats. Animals were dark-adapted for over 12 h before the ERG recordings. Anesthesia was achieved with an intramuscular injection of ketamine hydrochloride (36 mg/kg) and xylazine hydrochloride (1.6 mg/kg) (9:1), and the corneas were anesthetized with oxybuprocain hydrochloride (0.4%). Then, the pupils were fully dilated with tropicamide (0.5%) and phenylephrine (0.5%). The body temperature was maintained at 38°C with a heating pad (BWT-100; Bio Research Center, Nagoya, Japan) while recording. The head was affixed to the heating pad and placed in a Ganzfeld stimulator so that all eyes were examined in the same position and stimulated by the same flash energy.

Photostimulator

Light stimuli were emitted from a xenon arc lamp (YM-1; Sanso Seisakusho, Tokyo, Japan) to a Ganzfeld stimulator, and the duration was regulated by an electromagnetic shutter. We applied a single white flash for 50 ms, and the intensity, determined by photometry (J17; Sony/Tektronix, Tokyo, Japan), was 110 mW/m^2.

Recording System

An active needle electrode was placed on the cornea, an inactive reference electrode was subcutaneously positioned at the center of the scalp, and the ground electrode was subcutaneously implanted at the root of the neck. All electrodes were composed of tungsten. The ERG voltages were amplified (MEG-6108; Nihon Koden, Tokyo, Japan) and the signals were digitized (MacLab4s; ADInstruments Japan, Nagoya, Japan) using the software Scope 3.5 s (ADInstruments) and stored on a personal computer (Power Macintosh 7200; Apple Computer, Cupertino, CA, USA). Potentials were processed with a high cut at 1 kHz and a low cut at 50 Hz for OP recording, and at 0.5 Hz for a-wave and b-wave recording.

Measurement of Parameters

The Scope 3.5 s software measured all parameters. The amplitude of the a-wave was measured from the baseline to the trough of the a-wave. The amplitude of the b-wave was measured from the a-wave trough (Fig. 1a). The OP amplitudes were measured from a baseline drawn between successive troughs of wavelets (Fig. 1b). The implicit time was measured from stimulus onset to peak amplitude. We measured a-wave latencies from stimulus onset as described[12] to evaluate photoreceptor function (Fig. 1a). To evaluate the influence of retinal ischemia, we calculated the following ratios: b-wave amplitude/a-wave amplitude (b/a), O1 amplitude/a-wave amplitude (O1/a), and O2 amplitude/a-wave amplitude (O2/a). All data are presented as means ± SD. Results were statistically analyzed between normal control and diabetic groups using two-way repeated-measures analysis of variance (ANOVA) and the unpaired t test.

Results

The blood glucose levels of GK rats remained high from 4 to 48 weeks of age (Fig. 2), and cataracts as well as visible DR were absent. The a-waves, b-waves, and OPs were

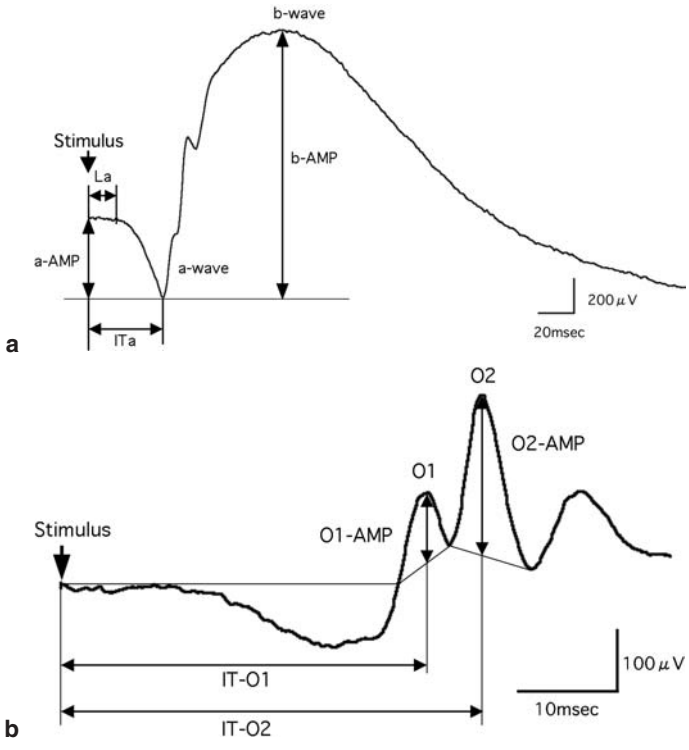

Figure 1. Procedure for measuring amplitude (*vertical arrows*), implicit time, and wave latency (*horizontal arrows*) of electroretinogram (ERG) a- and b-wave components (**a**) and oscillatory potentials (**b**) *AMP*, amplitude; *IT*, implicit time; *La*, a-wave latency.

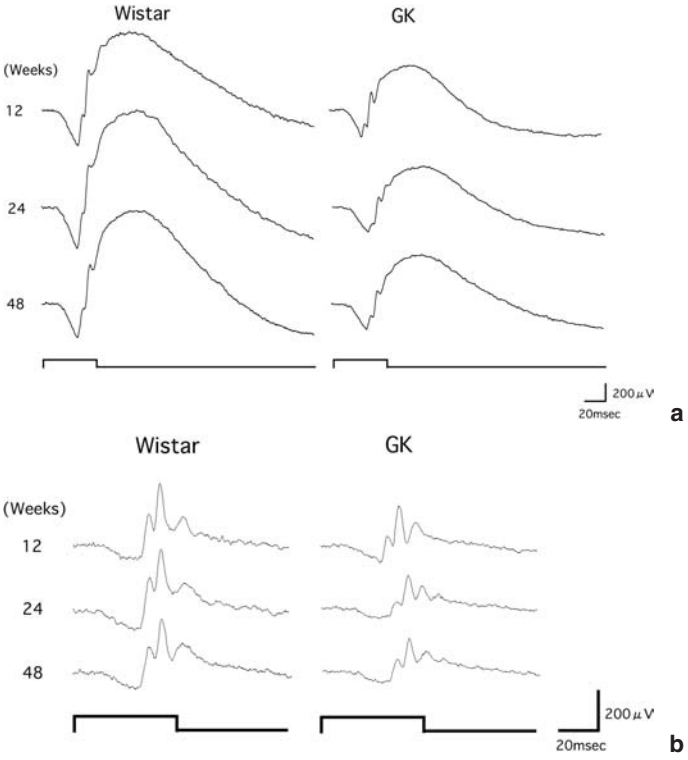

Figure 3. Sample ERGs recorded with a 0.5 Hz–1 kHz (**a**) and 50 Hz–1 kHz (**b**) band pass, in Wistar (*left*) and GK (*right*) rats at various ages.

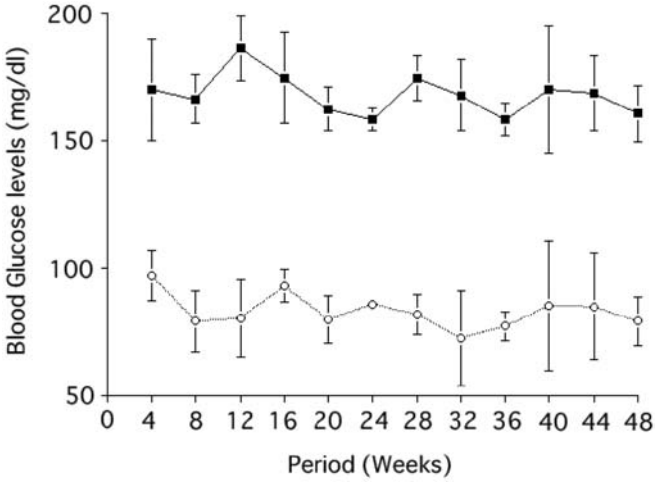

Figure 2. Relationship between duration of diabetes and blood glucose level in Goto-Kakizaki (GK) (■) and Wistar (○) rats. Points and error bars represent mean and SD, respectively. Blood glucose levels were significantly higher in GK than in Wistar rats ($P < 0.01$).

observed in all rats in time-dependent courses of ERG recordings. However, we compared only O1 and O2 between GK and Wistar rats, although three OPs were evident in some rats and four OPs were obvious in others (Fig. 3).

Time-Dependent Courses of ERG Recording

The amplitude of a-waves was reduced in 4-week-old GK rats and remained diminished at 48 weeks of age. However, time-dependent changes did not differ with age between Wistar and GK rats (Fig. 4a). The implicit times of the a-wave did not significantly differ (Fig. 5a), but the latencies of the a-wave were significantly increased in GK rats (Fig. 5b). The amplitudes of the b-wave were already decreased in 4-week-old GK rats, but time-dependent changes did not differ with age (Fig. 4b). The b/a ratios of GK rats were smaller than those of Wistar rats ($P < 0.01$).

The amplitudes of O1 and O2 were significantly diminished in 4-week-old GK rats and remained low for up to 48 weeks. The amplitudes of O1 and O2 in normal control rats decreased with age, and only the O2 amplitudes decreased with age in GK rats. However, the O1 amplitudes did not decrease with age in GK rats (Fig. 6), and the implicit times of O1 and O2 were not prolonged in GK rats (Fig. 7). The O1/a and O2/a ratios of GK rats were smaller than those of Wistar rats (O1/a, $P < 0.05$; O2/a, $P = 0.055$).

Fourteen-day-old Group

The a-wave amplitudes were significantly smaller in GK than in Wistar rats (Fig. 8).

Jpn J Ophthalmol
Vol 50: 211–216, 2006

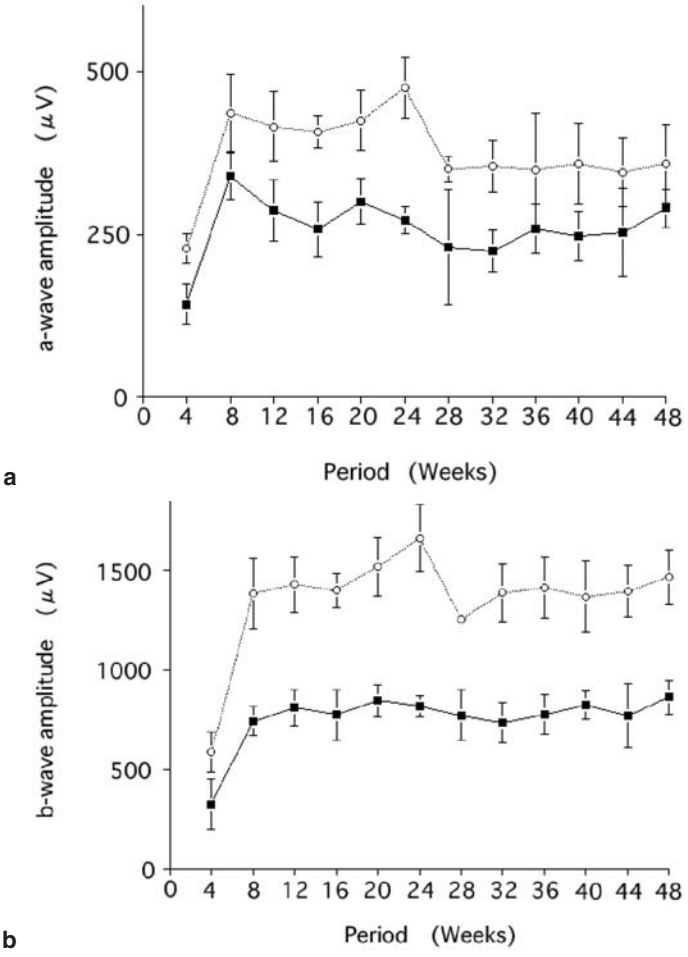

Figure 4. Averaged a-wave (**a**) and b-wave (**b**) amplitudes in relation to age. Points and error bars represent mean and SD, respectively. **a** Although GK (■) and Wistar (○) rats differed significantly ($P = 0.017$), the trend was similar between groups as they aged, but without statistical significance. **b** Although the difference between GK (■) and Wistar (○) rats was statistically significant ($P = 0.037$), the trends became similar as they aged.

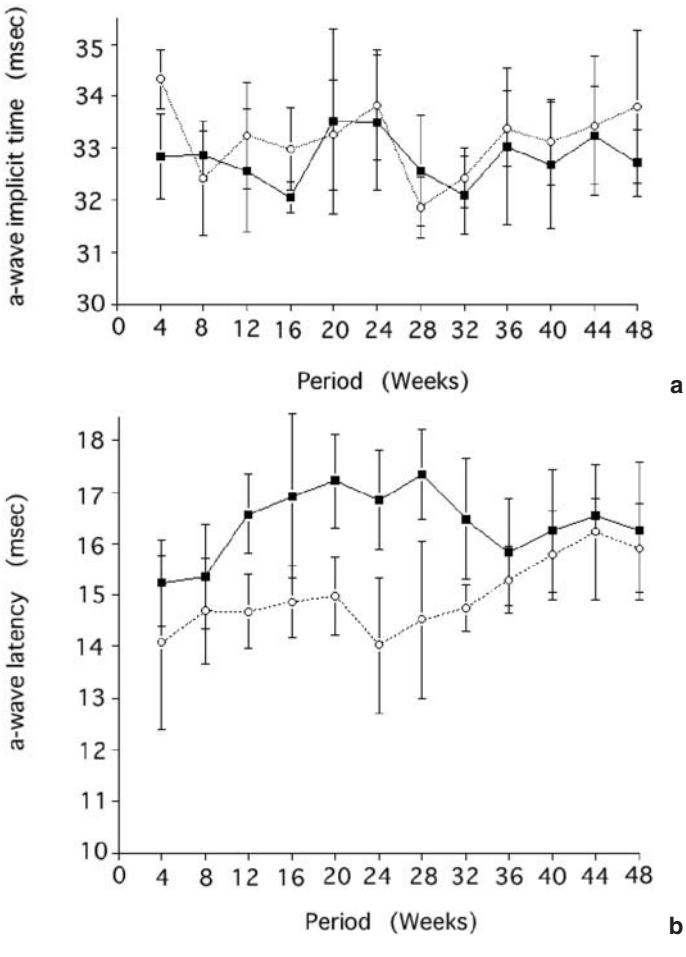

Figure 5. Averaged implicit times (**a**) and latencies (**b**) of the a wave relative to age. Points and error bars represent mean and SD, respectively. **a** Implicit times did not significantly differ between GK (■) and Wistar (○) rats at any age ($P > 0.05$). **b** Although a-wave latencies between GK (■) and Wistar (○) rats differed significantly ($P < 0.01$), the trends continued without statistical significance between groups as they aged.

Discussion

Although DR was not observed in any GK rats, the amplitudes of a-wave, b-wave, and OPs were already decreased at 4 weeks of age and remained diminished at all times examined. These findings indicated that the generation of the a-wave was suppressed at 4 weeks of age, and, consequently, the b-wave and OPs were also decreased, or that the generation of all waves was suppressed. We initially evaluated photoreceptor function in the GK rat. Others have determined a-wave latency solely as photoreceptor activity, since a-wave latencies are prolonged by damage to photoreceptors.[13]

However, in the present study, we found that the a-wave latencies were prolonged even in 4-week-old GK rats, indicating that photoreceptors were already the functionally disordered by this age. Several reports have described the functional disorder of photoreceptors caused by hyperglycemia or inheritable degeneration in diabetic rat models. For example, guanosine triphoshate (GTP)-binding activity is significantly decreased in rod outer segments (ROS) of the diabetic rat model, and G protein in ROS might be functionally inactivated as early as 2 weeks after the onset of diabetes. Moreover, GTP binding activity is normalized by insulin.[14]

On the other hand, in another study, a-wave amplitude was not significantly reduced at 2 weeks from the onset of hyperglycemia in rats with STZ-induced diabetes.[15] We found here that the amplitudes of all ERG components were decreased in GK rats even at 4 weeks of age, which was only 2 weeks after glucose intolerance is usually confirmed.

In addition, the a-wave amplitudes of GK rats were smaller than those of Wistar rats at 14 days of age. This finding indicates that photoreceptor abnormalities had

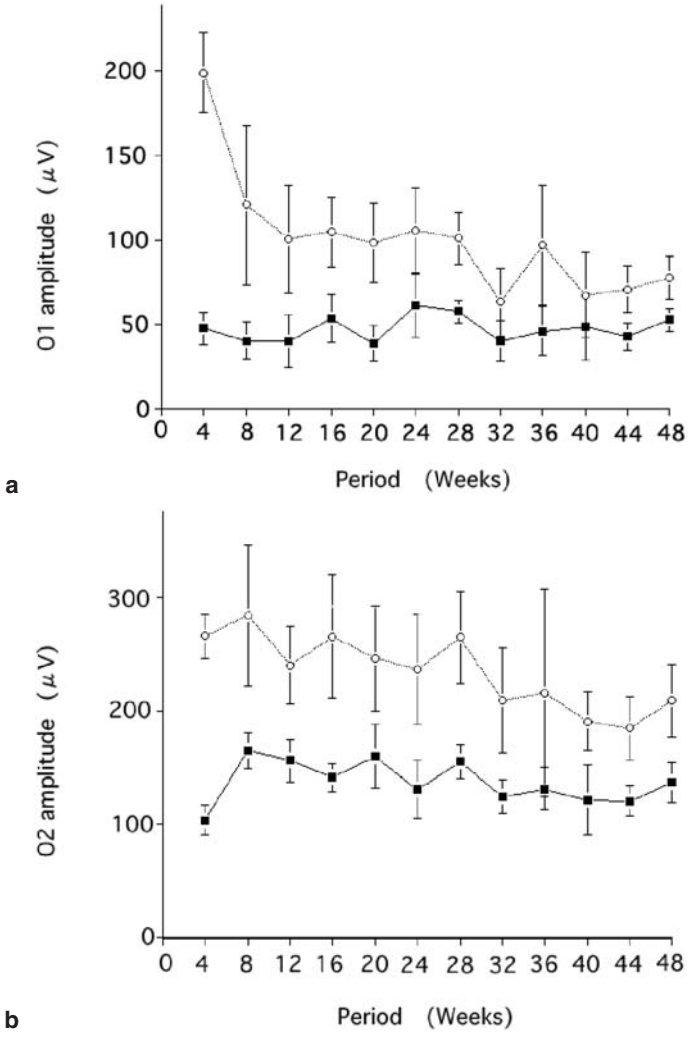

Figure 6. Averaged amplitudes of O1 (**a**) and O2 (**b**) of ERG relative to postnatal age. Points and error bars represent mean and SD, respectively. **a** O1 amplitude of the GK (■) rat was significantly lower than that of the Wistar (○) rat ($P < 0.01$). The trend differed statistically between groups with age ($P < 0.01$). Amplitude of Wistar rats declined with increasing age, whereas that of GK rats remained unchanged. **b** O2 amplitude of GK (■) rats decreased significantly compared with that of Wistar (○) rats ($P < 0.01$). The trend continued with age, without statistical significance between groups.

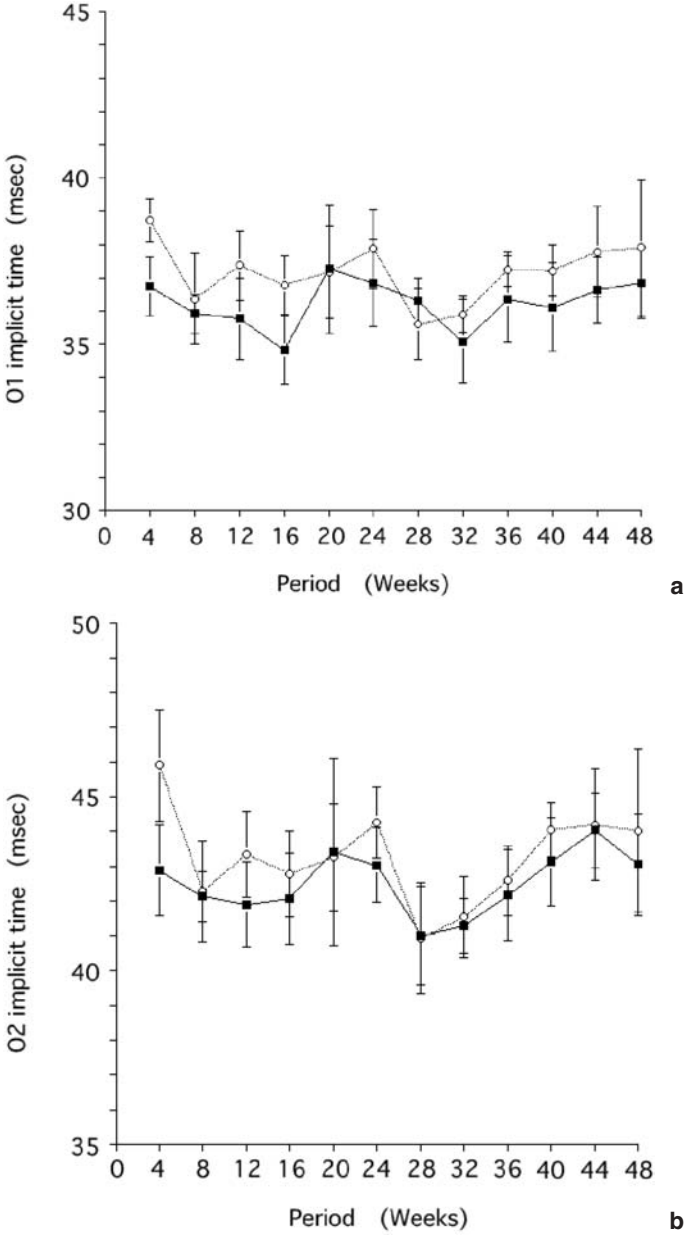

Figure 7. Averaged implicit times of O1 (**a**) and O2 (**b**) relative to postnatal age. Points and error bars represent means and SD, respectively. Implicit times of O1 and O2 did not differ significantly between GK (■) and Wistar (○) rats (both $P > 0.05$).

already developed in the young GK rat. Thus, we surmised that this functional change of photoreceptor was caused not by hyperglycemia but by inheritable degeneration without an apparent histological abnormality of the retina.[16] As a result, the amplitudes of the a- and b-waves were reduced at 4 weeks of age. Disorders of not only the inner retina but also the outer retina affect the generation of OPs. Thus, we supposed that OP amplitude was also reduced because of the photoreceptor disorder.

Although the a-wave latencies of GK rats were increased, a-wave implicit times did not differ between GK and Wistar rats. The reason for this difference is obscure. However, the a-wave of a standard ERG is a compounded waveform of three basic ERG components (PI, PII, and PIII). The a-wave implicit time was longer than a-wave latency in the ERG time course, and it would be more affected by the compounding of the ERG components. We therefore supposed that reflection of a small difference caused only by a photoreceptor abnormality in the a-wave implicit time might be difficult.

Another factor involved in DR pathogenesis is tissue hypoxia,[17] which might be caused by blood abnormalities. Retinal circulation time is significantly prolonged in the GK rat, and retinal blood flow is reduced at an early age without apparent retinopathy.[18] Furthermore, models of retinal

Jpn J Ophthalmol
Vol 50: 211–216, 2006

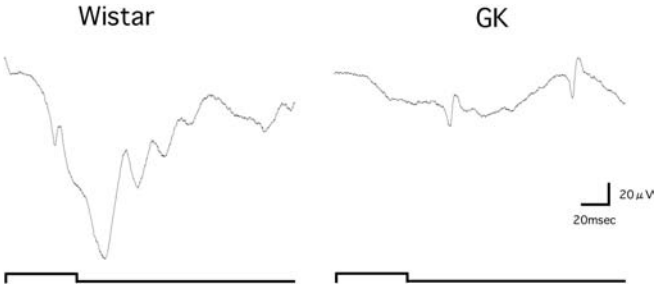

Figure 8. Sample ERGs recorded in Wistar (*left*) and GK (*right*) rats at 14 days of age (25.1 ± 0.7 μV vs. 143 ± 17.6 μV; unpaired *t* test, $P = 0.011$). Although electrocardiograms were recognized on these sample ERGs, the a-wave amplitude was smaller in GK than in Wistar rats.

hypoxia develop morphological and neurochemical changes.[19,20,21] Ischemic changes during diabetes in the GK rat alter the distribution of amino acid neurotransmitters[16] in a manner similar to that in experimental ischemic models, leading to the accumulation of glutamate and γ-aminobutyric acid (GABA).[19,20] The present b/a, O1/a, and O2/a results indicated that ischemia is induced in the GK rat retina. In general, OPs appear to be highly sensitive to disturbances of the retinal circulation,[11] and the implicit times of OPs are prolonged even in the absence of vascular dysfunction. We found that the implicit times of O1 and O2 were not prolonged. Therefore, our results indicate that retinal hypoxia in the GK rat was not severe enough to affect the implicit times and amplitudes of OPs. Even though hypoxia altered amino acid localization, OP amplitudes were reduced not by retinal hypoxia but rather by an inheritable photoreceptor dysfunction.

These results differ from those obtained from other diabetic model rats such as the STZ-induced rat (Type I model)[22] and the OLETF (Otsuka Long-Evans Tokushima Fatty) rat (Type II model).[23] Since the diabetic state is milder in the GK rat than in other rat models, retinal hypoxia of the GK rat would also be milder. As a result, the OP implicit times might not be altered, unlike those in other rat models. Since the WBN/Kob spontaneously diabetic rat has inheritable retinal degeneration,[24] whether an inheritable photoreceptor abnormality is induced in the GK rat should be investigated using other methods.

In conclusion, photoreceptors of the GK rat retina might be functionally disordered by inheritable degeneration, and, as a result, the amplitudes of its ERG components were already reduced at an early age. Although the retinal hypoxia caused by hyperglycemia might damage the GK rat retina, it might not be sufficient to disturb OP generation.

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