Evidence for Early Impairment of Macular Function With Pattern ERG in Type I Diabetic Patients

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The electroretinogram (ERG) elicited by alternating gratings at constant mean luminance (pattern ERG) is a focal response reflecting the activity of the directly stimulated retinal area. In addition, pattern ERG is related, unlike the flash ERG, to ganglion cell activity. Therefore, this technique may be used to evaluate the integrity of inner retinal layers in the macular region. In this study, the steady-state pattern ERG, in response to alternating gratings (1.7 cycles/deg spatial frequency; 9° field size) temporally modulated at 8 Hz, was recorded in 42 type I (insulin-dependent) diabetic patients with zero to four microaneurysms on fluorescein angiography and a duration of disease <11 yr. No patient had concomitant ocular or systemic complications. Mean pattern-ERG amplitude was significantly reduced in patients compared with age-matched control subjects (analysis of variance, $F = 25.6$, $P < 0.0001$). Significant differences were observed between control and diabetic subjects without retinopathy (Scheffé F test, $P < 0.0001$), between control and retinopathic subjects (Scheffé F test, $P < 0.0001$), and between diabetic patients without retinopathy and those with early retinopathy (Scheffé F test, $P < 0.02$). Pattern-ERG amplitude was inversely correlated with duration of diabetes ($r = 0.22$, $P < 0.05$). Our results suggest a macular dysfunction in early diabetes resulting from metabolic and/or vascular injuries in the neurosensory retina. *Diabetes Care* 13:412–18, 1990

Diabetic patients may show abnormal visual function that precedes or develops closely with clinically detectable retinopathy. Visual deficits in diabetes mellitus can be demonstrated by psychophysical and electrophysiological techniques. Psychophysical tests include contrast sensitivity (1,2), color vision (3), and recovery of cone function after exposure to bright light (4). Electrophysiological tests employ visually evoked retinal (electroretinogram; ERG) and cortical (visually evoked potentials) electrical responses (5–9). The results obtained with these techniques suggest a dysfunction of the central retina (i.e., the macula) that may occur in early diabetes. The macula, which has high neuronal density compared with the more peripheral retina, has the most significant retinal functions, e.g., visual acuity, contrast sensitivity, and color vision. According to experimental data, the primary site of metabolic and/or vascular damage in the diabetic retina is located in the inner retinal layers (8). Macular dysfunction in diabetes may therefore reflect a specific impairment of neural retinal elements (bipolar and ganglion cells and interplexiform and amacrine cells).

The ERG evoked by black-and-white bars or checkerboards, alternating at a constant mean luminance (pattern ERG; P-ERG), has been used clinically to evaluate macular function. The P-ERG is a focal response that reflects the activity of directly stimulated retinal areas (10) and is thought to be correlated to ganglion cell activity, unlike the more conventional flash ERG (11,12). The P-ERG technique may therefore provide a direct and objective index of inner retina function at the macula.

Great attention has been directed to the effects of diabetes on the nonvascular retina (13). We used the P-ERG approach to evaluate macular function in type I

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(insulin-dependent) diabetic patients with no or minimal signs of retinopathy.

RESEARCH DESIGN AND METHODS

Subjects. Forty-two patients with type I diabetes participated in this study. Thirty-two patients (64 eyes) showed no retinopathy, and 8 patients (16 eyes) showed few microaneurysms (<5) in both eyes, whereas in one participant, microaneurysms were seen in only one eye. The current metabolic control status was estimated by HbA_{1c} concentrations (7.2 ± 2.4% in our patients). Thirty-nine healthy subjects composed the control group (Table 1).

Before inclusion in the study, all subjects received a general ocular examination including direct and indirect ophthalmoscopy and slit-lamp biomicroscopy. Color fundus photography and fluorescein angiography of both eyes were performed for each patient. None of the patients had other ocular or systemic diseases or were receiving medications. Overall retinopathy severity was categorized according to the second level of the Klein classification (14). No macular edema was found in any subject. Subjects studied had a best-corrected visual acuity of 20/20 or better. Refractive errors, when present, were below ±1.00 diopters and were fully corrected during the test. Diabetic patients were tested after eating to avoid hypoglycemia. Informed consent was obtained from each subject after the nature of the test and the study were fully explained.

Methods. Fluorescein angiography was performed with a Kowa (Pro 1 50°) fundus camera after rapid injection of 5 ml of 10% fluorescein sodium into the antecubital vein. Angiograms were taken with ASA400 black-and-white film and graded according to a standard protocol previously described (14).

In the testing apparatus, sinusoidal gratings consisting of alternating light and dark bars were employed to generate the P-ERG (Fig. 1). The spatial frequency (i.e., *n* of light and dark cycles/unit of visual angle) was 1.7 cycles/deg (fixed contrast, 84%) electronically generated on a high-resolution television monitor and temporally modulated (sinusoidally) in counterphase at a temporal frequency (i.e., *n* of stimulus cps) of 8 Hz (Fig. 2). Mean luminance of the stimulus was held constant at 84 cd/m^2. The monitor was surrounded by a large piece of equiluminant white cardboard (70 × 70 cm) in a way that left a central square of the monitor uncovered (15). The employing field subtended a visual angle of 9° at 43 cm viewing distance. All subjects were able to maintain fixation on a black mark placed in the center of the stimulating field. Pupils were natural and previously measured (pupil sizes between control subjects [3.70 ± 0.6 mm] and diabetic patients [3.82 ± 0.5 mm] were not different). P-ERG was monocularly recorded by means of a skin electrode taped on the lower eyelid of the stimulated eye. An equal electrode placed over the eyelid of the unstimulated eye was used as a reference (interocular ERG) (10). Retinal signals were band-pass filtered between 1 and 30 Hz, amplified 100,000-fold, and averaged up to 800 responses (12-bit resolution; 0.5-ms sampling rate), allowing rejection of single sweeps disturbed by artifacts (blinks, eye movements, or head movements). Fourier analysis (Discrete Fourier Series; 16) of the averaged response was performed off-line to isolate the second harmonic component (i.e., twice the stimulation frequency) whose peak-to-peak amplitude in microvolts and phase in degrees were measured. We used the second harmonic amplitude instead of the peak-to-peak value of the original waveform because of its superior signal-noise ratio. In our experimental conditions, this component yielded the major contribution to the P-ERG (Fig. 3). Each test was repeated twice; the average amplitude variation between the two records was 5% for both control subjects and diabetic patients. The average noise at the second harmonic, obtained with the monitor covered by a piece of white cardboard, was 0.08 ± 0.04 μV.

A fasting blood sample to measure HbA_{1c} values was drawn from each patient in the morning. HbA_{1c} was assayed by a highly specific high-performance liquid chromatography method with 5.8% as the upper limit of the normal range.

Statistical analysis. Results are presented as means ± SD. Statistical evaluation of the data was performed with

TABLE 1
Clinical and metabolic data

Group	*n* (M/F)	Age (yr)	Age at diabetes onset (yr)	Duration of diabetes (mo)	HbA_{1c} (%)
Control	17/14	22.1 ± 6.1			
Insulin-dependent diabetes					
Normal fundus	13/20	20.1 ± 7.3	17.0 ± 7.4	37.6 ± 39.5	6.9 ± 2.1
Early retinopathy	4/5*	22.0 ± 4.3*	15.0 ± 4.3†	80.6 ± 38.5‡	8.7 ± 2.8§

Values are means ± SD.
*NS for comparison among the 3 groups.
†NS for comparison of the diabetic groups.
‡$P < 0.0001$ for comparison of the diabetic groups.
§$P < 0.005$ for comparison of the diabetic groups.

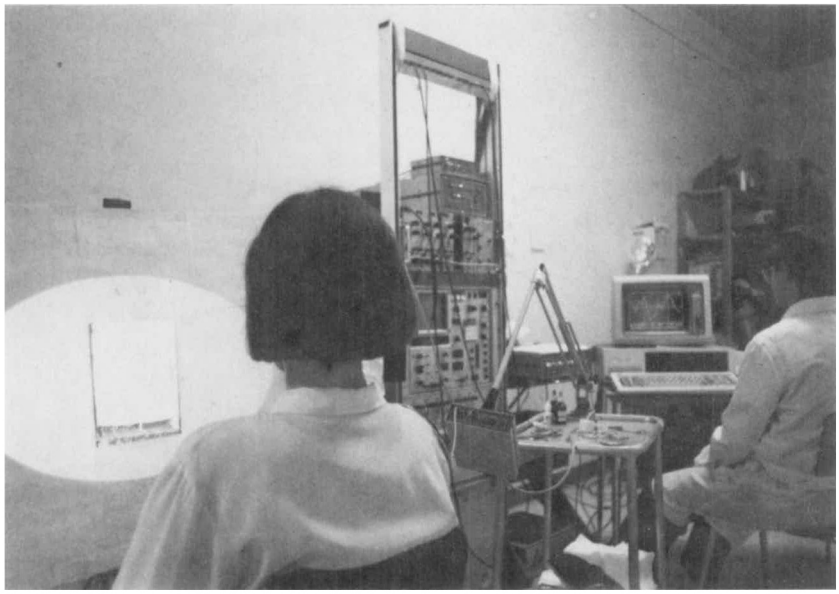

FIG. 1. Recording apparatus. *Left to right*, monitor (without 7 × 7-cm square placed over monitor) surrounded by white cardboard screen, amplifier, and computer used for data analysis.

the correlation between the two eyes of a subject, unpaired Student's *t* test, one-way analysis of variance (ANOVA), the Scheffé *F* test for multiple comparison, and linear regression analysis. Findings with an error probability <0.05 were considered statistically significant.

RESULTS

There was no significant difference between diabetic and control groups for sex and age at the time of study and between the two diabetic groups for age at onset of disease. Duration of diabetes was longer ($P < 0.001$) and HbA_{1c} values were higher ($P < 0.01$) in retinopathic patients than in the nonretinopathic group (Table 1). Figure 3*A* shows a representative example of steady-state P-ERG in response to 1.7 cycles/deg sinusoidal gratings obtained in a control subject. Figure 3*B* shows the Discrete Fourier Series of this response; the amplitude of each harmonic component is linearly proportional to the height of the vertical bars. The second harmonic response component (stimulus-reversal rate) yields the major contribution to the P-ERG. Table 2 shows the mean P-ERG amplitudes and phases in diabetic patients and in control subjects. Before statistical analysis of the results, we considered only the right eye amplitudes in control subjects, because the intereye correlation of the latter subjects was significant. On the contrary, the intereye correlation in our diabetic population was not significant ($r = 0.13$). We therefore used two-eye statistical analysis according to Ederer (17) and Ray and O'Day (18). The mean P-ERG amplitude was significantly reduced in diabetic patients (one-way ANOVA, $F = 25.6$, $P < 0.0001$) compared with control subjects. Significant differences were observed between control and diabetic subjects with no retinopathy (14.9, $P < 0.0001$) and between control and diabetic subjects with early retinopathy (21.5, $P < 0.0001$). The retinopathic patients had significantly lower mean P-ERG–amplitude values than those with normal fundi (4.4, $P < 0.02$). Figure 4 shows the individual P-ERG data in the three groups.

No significant differences in the mean phase values were observed between control subjects and diabetic patients. Linear regression analysis was performed with P-ERG amplitude as the dependent variable and with age at onset, duration of disease, and HbA_{1c} concentrations as the independent variables. A significant negative correlation of P-ERG amplitude with duration of diabetes was found ($r = 0.22$, df = 83, $P < 0.05$). The age at onset of disease and the HbA_{1c} values were not significantly correlated (Fig. 5).

DISCUSSION

Retinal function has been estimated in diabetes by electrophysiological methodologies, e.g., oscillatory potentials and light- and dark-adapted ERG with full-field stimulation (19,20). An abnormal electroretinal function may be detected in diabetic patients with various degrees of retinopathy (8). However, flash-ERG measurements reflecting the activity of the entire retina do not evaluate macular function. Similarly, oscillatory potentials may evaluate the hypoxia of overall retinal nonperfusion (8), but they do not directly estimate the inner retinal layer activity at the macula. The P-ERG could be useful in this respect (10). By means of P-ERG, Wanger and Persson (21) did not find functional changes in the retinas of diabetic patients with no or background retinopathy. Arden et al. (22) reported that P-ERG amplitude was significantly reduced only when cotton-wool exudates and angiographic evidence of

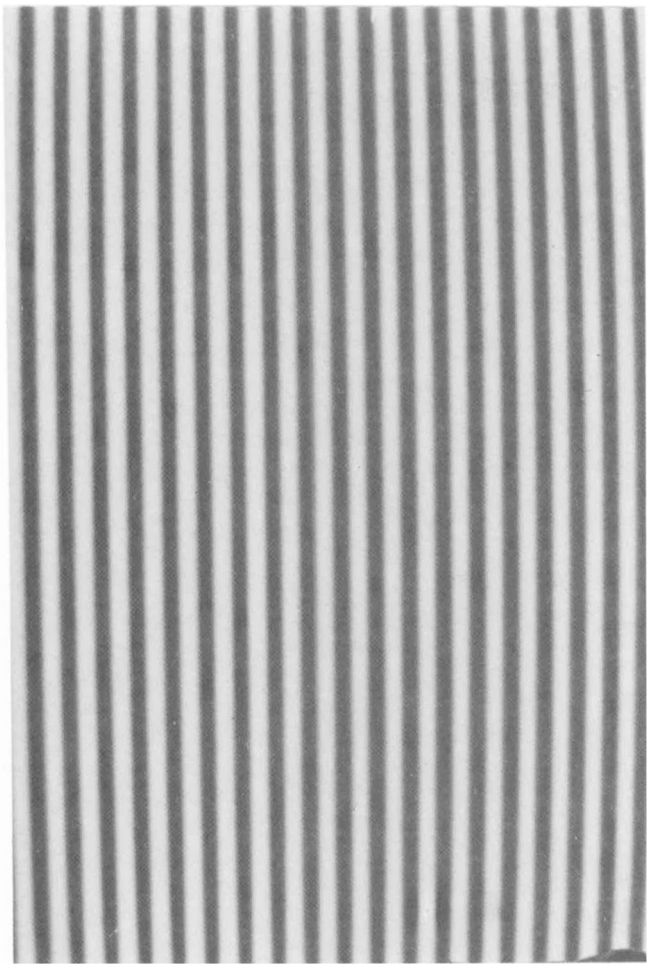

FIG. 2. Sinusoidal grating. Grating detection test of 1.7 cycles/deg spatial frequency (number of pairs of light and dark bars per degree of visual angle) is demonstrated. Patterned stimulus is electronically shown on television monitor.

capillary nonperfusion were present. Coupland (7) has also reported normal P-ERGs in type I diabetic patients without retinopathy, and he observed reduced P-ERG amplitudes only in patients with more than five lesions on graded fundus photographs (microaneurysms, dot and blot hemorrhages, focal areas of capillary dilation, or nonperfusion).

Our ERG data show that abnormal P-ERG responses may be observed in type I diabetic patients before the onset of a clinically evident retinopathy or after the appearance of small vascular retinal lesions. The difference between these results and those from other studies can be explained by different stimulation and/or recording techniques and by different criteria used in the evaluation of electrophysiological data. It has been demonstrated that under adequate stimulation and/or recording conditions, P-ERG may reflect the activity of inner retinal neurons (the ganglion cells themselves) (16). Previous studies have used slow-reversing checkerboards as a stimulus and analyzed the waveform (i.e., the amplitude of different components) of the resulting responses (7,21,22). We used sinusoidal gratings of high temporal frequency and Fourier analysis of the steady-state responses. In addition, other investigators have suggested that the use of a smaller field size of stimulation may be useful in reflecting the activity of the retinal ganglion cells (10,11,23).

The P-ERGs to slow-reversing checkerboards are complex responses in which the relative contribution of the outer retinal layers (i.e., photoreceptors) may be greater than that observed in the P-ERG evoked by high-frequency modulated sinusoidal gratings (24). Our most pertinent finding is the measurement of the second harmonic of the steady-state response, which may avoid the ambiguity of other recording methods in measuring

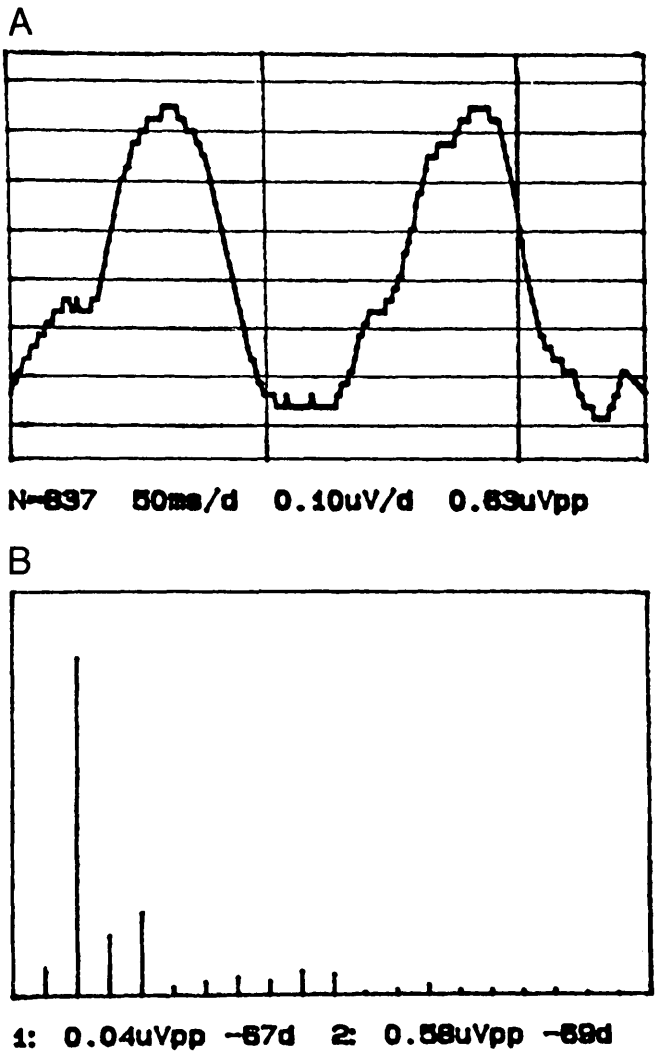

FIG. 3. *A*: representative example of steady-state pattern electroretinogram (P-ERG) in response to 1.7 cycles/deg at 8-Hz sinusoidal gratings obtained in control subject. *y*, 0.05 μV/div; *x*, 50 ms/div. *B*: Digital Fourier Series analysis of response. Note P-ERG is essentially dominated by 2nd harmonic component.

TABLE 2
Pattern-electroretinographic (P-ERG) results

Group	*n*	P-ERG amplitude (μV)	P-ERG phase (deg)
Control	39	0.53 ± 0.09	−103.6 ± 26.7
Insulin-dependent diabetes			
Normal fundus	67	0.41 ± 0.12	−99.9 ± 27.5
Early retinopathy	17	0.32 ± 0.13	−95.8 ± 30.3

Values are means ± SD.

the different components of the transient responses. Moreover, even though the absolute amplitude of the P-ERG recorded by a skin electrode is small, the signal-noise ratio may be favorable to record reliable responses. Finally, clinical investigations with an experimental procedure in which steady-state P-ERG has been employed have shown that in patients with optic neuritis or retinal ischemia, this procedure may reveal early functional losses in the inner retina (15,24).

Another interesting point is related to the different responses of the P-ERG amplitudes in the two eyes of the same diabetic patient. In many patients, we found clear neurophysiological abnormalities present only in one eye. For this reason, we chose the number of eyes studied and not the number of diabetic patients for our statistical analysis. In ophthalmic investigations, intereye correlation may be considered (17,18). In the natural history of diabetic retinopathy, each patient must have both eyes evaluated when the correlation coefficient between the two eyes is not significant. Conversely, if the intereye correlation is statistically significant, the analysis of both eyes can overestimate the precision of the results. In this study, we pooled the information from each eye of the individual diabetic patient as if it were obtained from two different subjects because of the nonsignificant intereye correlation.

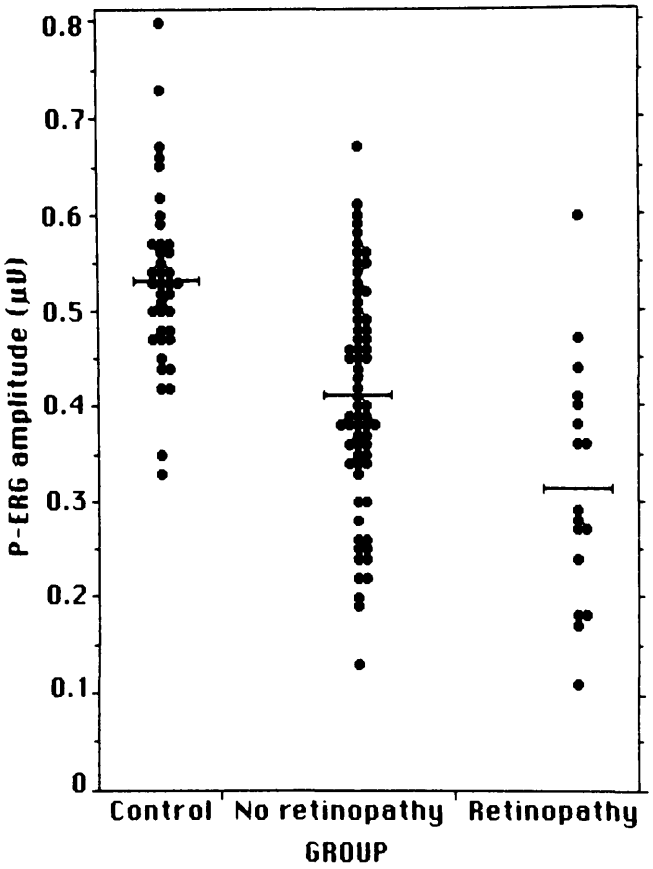

FIG. 4. Pattern electroretinogram (P-ERG) amplitude in each eye of control group (*n* = 39) and diabetic patients without (*n* = 67) and with (*n* = 17) retinopathy. *Horizontal bars* represent mean of each group.

Our results show that a P-ERG impairment can be observed in type I diabetic patients with no or minimal signs of retinopathy and with normal visual acuity. This fact suggests that in early diabetes a functional loss may occur in the inner retinal layers of the macular region. We also measured the phase angle of the second harmonic of the steady-state P-ERG. The phase angle is similar to the commonly measured latency-to-peak of transient evoked potentials (25). In our patient sample, a phase abnormality was observed in only a few patients with minimal retinopathy. Coupland (7) also did not find abnormal time-to-peak latencies of the transient P-ERG in type I diabetic patients.

The mechanisms responsible for causing dysfunctions in P-ERG amplitudes in type I diabetic patients remain uncertain. Hyperglycemia plays a key role in the development of retinopathy. Nevertheless, it has not been established whether these neurosensory deficits are directly due to the metabolic consequences of a persistent hyperglycemia of the retinal neurons or vessels. In this study, we found HbA_{1c} values higher in the retinopathic group. This evidence is insufficient to support the hypothesis that poor long-term glycemic control could contribute to the development of retinopathy. HbA_{1c} is only a good index of short-term metabolic control, because it does not indicate the glycemic status of patients in previous years. Patients with similar chronic hyperglycemia values can differ markedly in their susceptibility to diabetic retinopathy. The interval between the onset of hyperglycemia and the formation of the earliest microaneurysms is lengthy. Events occurring during this interval are indispensable to the pathogenesis of the ensuing retinopathy. The development of irreversible tissue damage in diabetic patients could be due to the combination of some sequelae of high glucose concentrations, e.g., excessive nonenzymatic protein glycosylation (26), abnormal aldose reductase activity and polyol production, gradual disappearance of retinal pericytes (27), and acute hemodynamic abnormalities in the retinal vasculature (28). Functional changes with potentially lasting effects on retinal capillary beds are produced early in this period.

In this study, we found a significant negative correlation between P-ERG amplitudes and duration of diabetes. This result is consistent with increasing evidence that the background lesions are associated with the du-

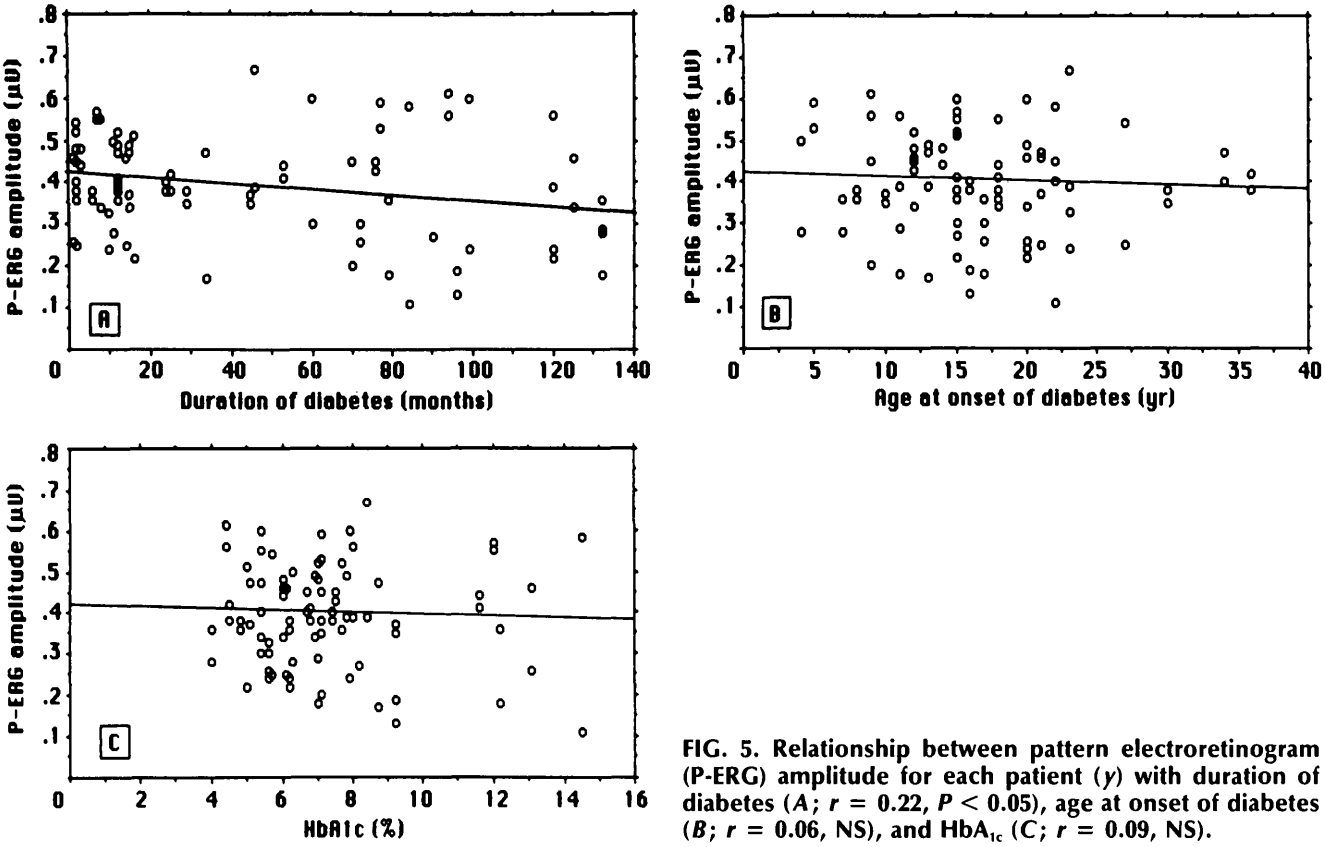

FIG. 5. Relationship between pattern electroretinogram (P-ERG) amplitude for each patient (*y*) with duration of diabetes (*A*; $r = 0.22$, $P < 0.05$), age at onset of diabetes (*B*; $r = 0.06$, NS), and HbA_{1c} (*C*; $r = 0.09$, NS).

ration of the disease, especially when studied over many years (29–32). However, the specific relationship between exposure and development of early retinopathy is unclear. The P-ERG amplitude, which progressively decreases during the course of diabetes, may indicate an increasing vulnerability to the development of retinopathy.

In conclusion, our results suggest that P-ERG may detect early functional deficits in type I diabetic patients in the presence of a normal fundus and normal visual acuity. In the macula, these abnormalities may be primitively localized in the ganglion cells of the retina. This technique may also contribute to understanding the pathogenesis of diabetic retinopathy. We have presented a P-ERG method in response to sinusoidal gratings consisting of alternating light and dark bars modulated at high frequency for its stimulus applicability to a clinical environment. However, we still do not know the advantages of P-ERG as a screening test. Longitudinal studies with P-ERG in type I diabetic patients may answer this question.

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