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Bimoclomol improves early electrophysiological signs of retinopathy in diabetic rats

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A silent process involving both neural and vascular structures in diabetic retina persists for several years before clinically detectable retinopathy. Recordings of the electroretinogram (ERG) and visual evoked potential (VEP) provide early warning of abnormalities in the visual pathway of diabetic patients and animal models. Treatment of streptozotocin-diabetic rats for 1 or 2 months with the heat-shock protein coinducer bimoclomol, a drug ameliorating experimental neuropathy, prevented and corrected the abnormal increase in latency and reduction of amplitude of ERG and VEP waves both in acute and chronic experiments. Improvements may be explained by cytoprotective effect of bimoclomol on retinal glia and/or neurons against diabetes-related ischemic cell damages. These findings suggest that bimoclomol may have future therapeutic use in diabetic retinopathy. *NeuroReport* **9: 2029–2033© 1998 Rapid Science Ltd.**

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Introduction

Diabetic retinopathy (DR), the leading cause of blindness, has traditionally been attributed to hyperglycemia-associated microangiopathy.[1] The process, initiated by the metabolic abnormalities of diabetes, is characterized by increased thickness of vascular basement membranes[2] and excess deposition of extracellular matrix components[3] leading to capillary non-perfusion, retinal ischemia and leakage.[4] However, an early retinal neurosensory dysfunction may also play a role in the development of DR according to the data from electrophysiological studies in both humans and animals.[5] In diabetic patients a functional loss of the innermost retina (ganglion cell and preganglion cell layers) has been detected by electroretinographic (ERG) and visual evoked potential (VEP) recordings before vasculopathy.[6,7] Spontaneously diabetic BB/W rats develop a central sensory neuropathy, characterized functionally by modification of VEP and structurally by dystrophy in the retinal ganglion cells and optic nerve fibers without visible vascular changes.[8] ERG components, especially the glial (Müller cells) originated b-wave and its independent neuronal wavelets, the oscillatory potentials (OPs), are highly sensitive to oxygen supply, blood flow and blood glucose.[9] Early diabetes reduces their amplitudes and delays the latencies, so these parameters are of clinical importance in diagnosis and prognosis of DR.[6,10,11] Aldose reductase inhibitors[12,13] and carnitine[14] are known to act mainly on ERG wave latencies in streptozotocin-induced diabetic rats. Bimoclomol, a new drug with heat-shock protein (HSP)-inducing activity,[15] protected against early retinal structural changes[16] and ameliorated peripheral neuropathy in diabetic rats.[17]

The aim of the present investigation was to examine the effects of preventive and curative treatments of bimoclomol on early electrophysiological signs of DR in rats. Diabetes was induced by injection of streptozotocin, and the drug's effect on retinal and cortical alterations was evaluated by measuring ERG and VEP parameters in anesthetized and freely moving rats.

Materials and Methods

Animal preparations: All experiments were performed using male Crl (WI) BR rats ($n \geq 6$/group, Charles River Reference Laboratory, SOTE, Hungary; 10–12 weeks old) in two separate, acute and chronic, experimental arrangements. Diabetes was induced by injecting 45 mg kg^{-1} streptozotocin (STZ, Sigma, St.Louis, MO, USA) in saline into the tail vein. Diabetes was verified 24 h later by estimating hyperglycemia (> 15 mmol l^{-1}, Glucofilm,

Ames, Diagnostics Div., USA). The total duration of diabetes was 2 months with bimoclomol treatment being either preventive or curative. Preventive treatments were started after confirmation of diabetes whereas curative treatment was initiated after 1 month of untreated diabetes. All animal treatments were in accordance with the recommendation of Guidelines on the Use of Living Animals in Scientific Investigations (US 1994).

Experiments on anesthetized rats: Retinal function was measured on five groups of rats. Three groups of five were treated orally: two received 20 mg kg^{-1} bimoclomol, preventive or curative, the third group received 50 mg kg^{-1} of aminoguanidine (Sigma, St. Louis, MO, USA), the reference, preventively. Non-diabetic and 2-month untreated, diabetic control groups were also included.

At the end of the treatment period, electrophysiological examination for detection of ERGs was carried out according to the method of Kozak *et al.*[13] Briefly, under anesthesia (Nembutal 60 mg kg^{-1}, i.p., CEVA, France), the left eye of dark-adapted (for 3 h) rats was stimulated by white flashes under weak red background illumination. By this modification and setting a 10 Hz filter to the amplifier, more pronounced OPs were evolved from the rod-type retina of rats. Five light impulses (12 ms with 15 s interstimulus interval) were delivered by a Monitron (RGB W-01 Hungary) photostimulator via a light emission diode (LED 5mm, LF-59, Conrad, Germany, emitting 100 mcd full color light) placed 1–2 mm from the cornea. ERGs were derived by 'one-point-touch' corneal surface electrodes positioned with a micromanipulator. The amplified (DC amplifier, Experimetria, Hungary) responses were stored on an IBM compatible PC for subsequent analysis.

The parameters of averaged ERGs were determined by Datawave (Technologies Co., USA) and Pti Delta 2.0 (Microsoft Co., USA) softwares. The amplitude of the a-wave was measured from the baseline to the trough of the a-wave. The amplitudes of OP2 and OP3 were measured from the nadir to the adjacent peaks. The wave latencies were determined from stimulus onset to the time of peak amplitude.

Experiments on conscious rats: Retinal and cortical functions were measured simultaneously in freely moving rats with chronic electrodes. Chronic implantation of electrodes was accomplished under halothane (1% in air) anesthesia using the method of Galambos *et al.*[18] Briefly, a multistrand wire electrode was set on the corneal surface for ERG recording. Stainless steel watchmaker screw cortex electrodes inserted into the fronto-parietal and occipital areas served to record electroencephalogram (EEG) and VEP. A small LED (Bright LED 5 mm, Electronics, Hungary) was implanted above the left eyeball for red light stimulation of high intensity (300 mcd). Insulated wires from the LED and electrodes were soldered to leads in a socket attached to the skull in the midline with dentacrylate layer.

Three groups of animals were used: one was implanted before diabetes induction to study the development of diabetic alterations in ERG and VEP; another group was implanted after diabetes induction but before starting the curative treatment with daily bimoclomol (10 mg kg^{-1}, i.p.). The time course of the curative effect was followed by serial measurements for 3 weeks. The treatment off effect was studied by using a 1-week washout period, when no treatment was applied. The remaining group was the implanted age-matched non-diabetic control.

From the 4th postoperation day experimental sessions in a chamber were performed weekly on dark-adapted (for 30 min) rats during synchronized EEG. Amplified (Grass EEG model 8B amplifiers USA) responses were recorded on a data collection system (Cambridge, UK; CED 1401). Averaging (25 times) was done by Sigavg 6.0 software of CED. Data were stored digitally for subsequent curve and data analysis.

The magnitude of the b-wave, the separated (filtered by Bessel filter) OPs, designated OP1, OP2, OP3 in order of superimposition on the b-wave and their sum (OP1-3), as well as the P1 component (the first positive deflection) of VEP were evaluated by measuring the subcurve areas (Fig. 1). The ERG and VEP were analysed by a selfdevised program written in Matlab for Windows (MathWorks Inc., USA)

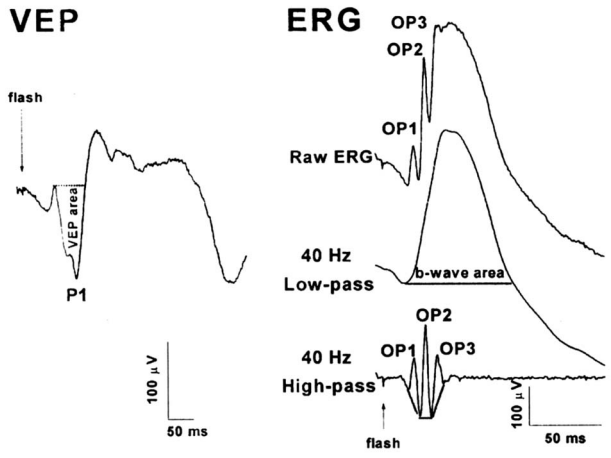

FIG. 1. Visual evoked potential (VEP) and electroretinogram (ERG) recorded from a non-diabetic conscious rat on red flash delivered by an implanted diode. The first positive wave (P1) of VEP was evaluated. The subcurve areas of the b-wave and oscillatory potentials (OP1–3) were determined after a low-pass and high-pass filtering the raw ERG.

software environment. The b-wave and VEP P1 latencies were measured as the intervals between the stimulus onset and the onset of the corresponding waves.

Statistical analysis: All data are presented as mean ± s.e.m. Student's *t*-tests, (unpaired and paired) and non-parametric Mann-Whitney U statistics were used (Graphpad Instat statistical package, San Diego, CA) at a significance level of $p < 0.05$. The beneficial effect of drug treatment on the diabetes-induced deficits (reduction in subcurve areas or increase in latencies) of ERG and VEP parameters was calculated on a percentage basis using the deficit produced by STZ-induced diabetes as 100%.

Results

Plasma glucose level following STZ administration was in the diabetic range (three-fold elevation) in animals treated with bimoclomol or aminoguanidine and in untreated animals. Diabetes caused a loss in weight gain over the time.

Effects of bimoclomol on ERG of anesthetized diabetic rats: STZ diabetes of 2 months duration resulted in impaired amplitudes and latencies of ERG waves. The depressed a-wave slope was ameliorated by bimoclomol (Table 1). The diminished amplitudes of OPs were improved by both types of bimoclomol treatment and aminoguanidine: by 54% ($p < 0.05$), 80% ($p < 0.01$) and 39% for OP2 and by 84% ($p < 0.05$), 122% ($p < 0.01$) and 44% ($p < 0.05$) for OP3. The a-wave peak latency remained unchanged, the others were lengthened significantly due to diabetes. The prolongation of peak latencies of OPs was normalized by preventive treatment with bimoclomol and was shortened by 74% ($p < 0.05$) for OP2 and by 84% ($p < 0.05$) for OP3, when given curatively. Aminoguanidine was less effective.

Effects of bimoclomol on ERG and VEP in freely moving diabetic rats: Weekly monitoring in the first group showed a gradual decrease in the magnitude of each OP, and their sum (OP1–3) with diabetes duration. OP1 and OP2 changed significantly from the first week of diabetes. The deficit reached 63% for OP1–3 by the end of month 1. The b-wave and VEP P1 areas, showing more variability, were reduced by 54% and 65%, respectively, $p < 0.05$ (Fig. 2) by this time. In this group the control values of ERG and VEP parameters before diabetes induction were analogous to those obtained in aged-matched non-diabetic control rats. In the second group similar reductions were measured after 1 month of untreated diabetes: 68% for OP1-3, 49% for b-wave and 59% for VEP P1 (all $p < 0.01$). Improvements of diabetic deficits by 51% for OP1-3, by 88% for b-wave and by 54% for VEP P1 (for

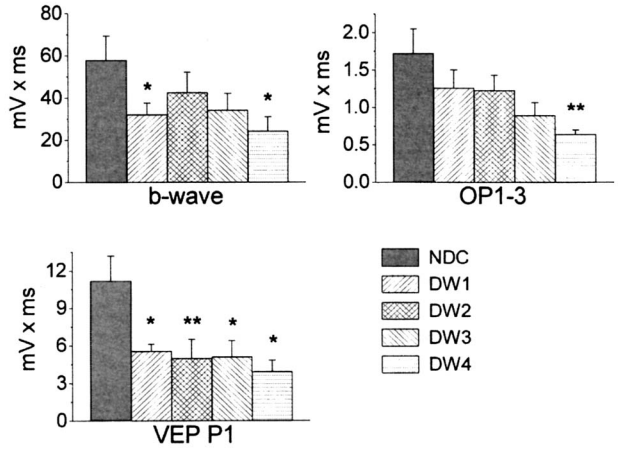

FIG. 2. Effects of STZ-induced diabetes on ERG and VEP parameters defined by weekly monitoring. Histograms show the mean (± s.e.m.) subcurve areas (mV × ms) of the b-wave, the sum (OP1–3) of oscillatory potentials OP1, OP2, OP3, and the first positive component (P1) of VEP before (non-diabetic control, NDC) and 1, 2, 3, and 4 weeks after diabetes-induction (DW1–4). *$p < 0.05$, **$p < 0.01$ compared to NDC in selfcontrolled paradigm.

Table 1. Effect of bimoclomol on ERG alterations in streptozotocin-diabetic rats

			Diabetic treated (p.o.)		
			Bimoclomol 20 mg kg^{-1}		Aminoguanidine 50 mg kg^{-1}
ERG parameters	Non-diabetic untreated ($n = 7$)	Diabetic untreated ($n = 6$)	Preventive ($n = 6$)	Curative ($n = 7$)	Preventive ($n = 5$)
a-wave slope (μV ms^{-1})	−29.0 ± 3.1	−17.9 ± 3.5^{+}	−25.0 ± 0.9	−28.8 ± 1.6*	−20.9 ± 2.7
OP2 amplitude (μV)	863.6 ± 46.9	458.7 ± 76.7^{+++}	677.6 ± 17.5*	782.3 ± 62.5**	617.5 ± 55.5
OP3 amplitude (μV)	908.9 ± 33.9	558.0 ± 63.7^{+++}	852.6 ± 73.9*	984.9 ± 100.8**	713.8 ± 104.2*
OP2 latency (ms)	28.3 ± 0.4	32.1 ± 0.9^{++}	29.3 ± 0.6*	27.9 ± 0.7**	30.3 ± 0.5
OP3 latency (ms)	39.2 ± 0.7	44.9 ± 1.6^{++}	40.1 ± 0.9*	38.3 ± 1.2*	43.6 ± 2.6

OP2, OP3: oscillatory potentials; Values are expressed as mean ± s.e.m.
$^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$ *vs* non-diabetic control; *$p < 0.05$, **$p < 0.01$ *vs* diabetic control.

all $p < 0.05$) were achieved after a 3-week curative treatment (Fig. 3). Following a washout period of 1 week, responses declined close to the diabetic level. The b-wave and VEP P1 latency changes could be demonstrated only 1 month after diabetes induction with prolongations of 30% and 23%, respectively, ($p < 0.01$). Bimoclomol shortened the latencies by about 48% ($p < 0.05$) and 19%, respectively (Fig. 4).

Discussion

In the present study, the preventive and curative effects of bimoclomol on DR were investigated in both anesthetized and freely moving rats. The freely moving animals had chronic electrodes which allowed us to follow the development of visual disturbances defined by ERG and VEP recordings without anesthetic interaction. Our data provide the first evidence that the amplitude alterations of ERG and VEP P1 begin 1 week after STZ injection and progress week by week reaching a stable reduction by the end of the first month of diabetes. The b-wave and VEP P1 latency changes, however, were significant only after 1 month of diabetes. Diabetes also depressed amplitudes and increased latencies of ERG waves in anesthetized rats. The abnormalities of ERG and VEP were prevented and corrected by bimoclomol treatment.

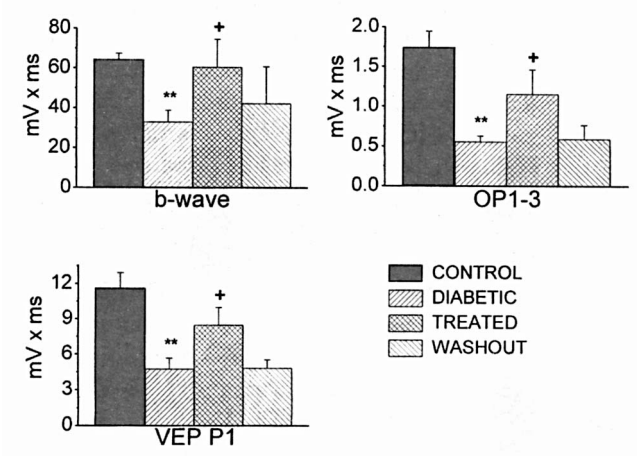

FIG. 3. Curative effects of bimoclomol treatment (10 mg kg^{-1}, i.p., for 3 weeks) on diabetic abnormalities of ERG and VEP parameters in conscious rats. Bars show mean (± s.e.m.) subcurve areas (mV × ms) of the b-wave, OP1-3, and VEP P1 in non-diabetic control as well as in untreated and treated diabetic groups. Washout (for 1 week) illustrates the treatment off effect. $^{**}p < 0.01$ *vs* non-diabetic control; $^{+}p < 0.05$ *vs* untreated diabetic control.

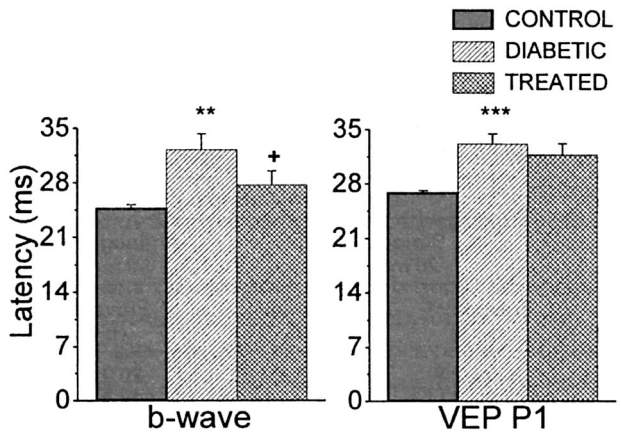

FIG. 4. Effect of bimoclomol on diabetes-related latency prolongations (ms) when administered i.p. to 1 month untreated diabetic rats over 3 weeks. Each bar represents mean ± s.e.m. $^{**}p < 0.01$, $^{***}p < 0.001$ *vs* non-diabetic control; $^{+}p < 0.05$ *vs* untreated diabetic control.

Diabetic changes of ERG components of both neuronal and glial origin were influenced by bimoclomol. The drug partially improved the depressed a-wave slope seen in diabetic rats. The a-wave is a gauge of photoreceptor response. Bimoclomol completely prevented the prolonged peak latencies at OP2 and OP3 components and restored close to normal level their diminished amplitudes in anesthetized animals. Similar results were found in freely moving rats as early as 3 weeks after the start of treatment. Bimoclomol treatment promoted the appearance of OP4, which was previously invisible in anesthetized non-treated diabetic rats. Normal activity of bipolar, amacrine and ganglion cells and the optic nerve is essential for generation of OPs.[10] Recovery of diabetic deficiencies of ERG and VEP P1 by bimoclomol was reversible after a 1-week washout period. Bimoclomol proved to be highly potent against diabetic changes of the b-wave, the only signal of glial origin. In diabetes mellitus, Müller glial cells are one of the first cells of the retina to show pathology. Interdigitating between the various retinal layers, they control and modulate neuronal activity and participate in defending the retinal function against ischemia,[19] perhaps by producing stress proteins.[20] The increase of VEP P1 latency, indicating the conduction slowing along the large myelinated fibres of optic nerve, can be considered as the consequence of an early neuropathy of central visual pathways.[12] Sima and co-workers[8,12] have previously reported that similar VEP latency changes appeared to be parallel with retinal ganglion cell dystrophy and optic nerve axonopathy in 6-month diabetic BB/W-rats.

Hyperglycemia-driven mechanisms such as non-enzymatic glycation have been linked to increased vascular permeability, compositional changes of basement membrane and endothelial cell damage leading to microvascular obliteration and retinal ischemia.[1,3,4] OPs display a peculiar sensitivity to the overall inner retinal ischemia, due to impaired blood supply, which can be used in the diagnosis of DR as a quantitative measure.[9,10] The early reduction of OP amplitudes correlates with circulatory deficiency in the retina.[11]

The delay of wave latencies in diabetic subjects correlates with basement membrane thickness,[13] indicating the stage of DR.[3] While aminoguanidine, a pharmacological inhibitor of glycated protein formation,[21] had similar activity to that of bimoclomol in reducing increased permeability in our previous study,[16] it was less effective in preventing early electrophysiological abnormalities of ERG when used as a reference in the present experiment.

An early factor in DR pathogenesis is tissue hypoxia,[1,4] which may be caused by reduced erythrocyte perfusion in the innermost capillary bed.[22] The neuroretinal abnormalities defined by ERG are associated with the same part of the retina.[6–8,10,12] Proteins considered to be involved in the recovery of neuronal damage after ischemia include immediate early gene products, stress response proteins such as HSPs, and neurotrophic factors.[23] The glycation of the proteins participating in the defense system of the cell are responsible for integrity of the molecular chaperons (such as α-crystallins) could even drive a vicious circle in the development of diabetic complications.[24] In our previous investigations bimoclomol maintained the structural integrity of the basement membrane and endothelial lining of the retinal capillaries,[16] improved peripheral diabetic neuropathy,[17] protected against cerebral vasogenic damage[25] and induced production of cytoprotective stress proteins.[15] Based on these data, it is hypothesized that bimoclomol ameliorates DR and neuropathy through its beneficial action on the maintenance and restoration of the normal integrity of capillary barrier function, on one hand. On the other hand, bimoclomol could improve the function of the innermost retina perhaps by decreasing the glial vulnerability and/or by promoting restorative processes of Müller glia.

Conclusion

Bimoclomol prevents and corrects early retinal neurosensory dysfunctions defined by ERG and VEP in STZ-diabetic rats. Improvements demonstrate the reduction of severity of retinopathy possibly by exerting cytoprotective effect on glia and neurons and/or protecting against basement membrane thickness of the retinal vessels which are known to correlate with the OP latencies. This corresponds to our previous histological results obtained in retinopathic rat models.

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