Glial and neuronal dysfunction in streptozotocin-induced diabetic rats

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Abstract Neuronal dysfunction has been noted very soon after the induction of diabetes by streptozotocin injection in rats. It is not clear from anatomical evidence whether glial cell dysfunction accompanies the well-documented neuronal deficit. Here, we isolate the Müller cell driven slow-P3 component of the full-field electroretinogram and show that it is attenuated at 4 weeks following the onset of streptozotocin-hyperglycaemia. We also found a concurrent reduction in the sensitivity of the phototransduction cascade, as well as in the components of the electroretinogram known to indicate retinal ganglion cell and amacrine cell integrity. Our data support the idea that neuronal and Müller cell dysfunction occurs at the same time in streptozotocin-induced hyperglycaemia.

Keywords Diabetes · Retina · Rat · Streptozotocin · Müller cell · Electroretinogram

Introduction

Diabetic eye disease is a leading cause of blindness in those of working age (aged 30–69 years) [1]. It is clear that chronic hyperglycaemia leads to changes in retinal blood vessels. The severity of blood vessel changes is dependent on the level and duration of hyperglycaemia. More recent clinical and laboratory research suggests that in addition to

vascular complications, glial and neuronal dysfunction can occur very early in the disease process [2, 3].

Studies of retinal function in diabetic patients suggest that functional changes are detectable before vascular changes manifest. For example, the high-frequency wavelets recorded on the ascending limb of the electroretinogram (ERG) b-wave (oscillatory potentials), thought to reflect inner retinal inhibitory circuits, are smaller in those with diabetes with no evidence of background diabetic retinopathy as defined using photography [4] or fluorescein angiography [5]. A reduction in amplitude of the response to a contrast reversing pattern checkerboard (pattern ERG) has also been observed in eyes that do not have overt clinical signs of retinopathy [6]. More recent studies using the multifocal ERG show that local timing delays can predict those patches of retina that will go on to develop non-proliferative diabetic retinopathy [7, 8]. Thus, in general, studies suggest that functional responses arising from post-receptor pathways, such as oscillatory potentials [9], the photopic negative response [10] and the visual evoked potential [9], decline before outer retinal responses in the course of diabetic eye disease. In agreement with the physiology, anatomical and imaging studies in human diabetes show that the loss of inner retinal neurons (in particular the retinal ganglion cells) and a thinning of the retinal nerve fibre layer can occur early in diabetes [11–13].

Recent laboratory studies have attempted to consider more closely the time course of hyperglycaemia-induced changes in neuronal, glial and vascular dysfunction. Many of these studies have been undertaken in the streptozotocin (STZ) rat model of type-1 diabetes. Neuronal dysfunction in terms of attenuated and delayed ERG responses occurs as early as 2 weeks after the induction of hyperglycaemia in rats [14, 15]. Moreover, inner retinal function (i.e. oscillatory potentials and scotopic threshold responses) is more

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sensitive to hyperglycaemia than is the outer retinal component in STZ-induced diabetes [16]. It is of interest that Li et al. [15] report that the ERG b-wave was reduced by 2 weeks after diabetes induction, which was well before a detectable increase in glial fibrillary acidic protein expression in Müller cell end feet (6 weeks). These data suggest that dysfunction of inner retinal neurons precedes glial changes in diabetic rats.

A previous study suggests that non-neuronal function in terms of the retinal pigment epithelial driven c-wave is reduced as early as 2 weeks after STZ-diabetes [17]. This is consistent with the finding that the retinal pigment epithelial driven electrooculogram [18] is sensitive to change in glucose level. These data raise the possibility that non-neuronal changes may manifest early in the course of STZ-diabetes. Thus, the aim of this study is to consider whether glial and neuronal dysfunctions are both found at 4 weeks following the induction of STZ-diabetes. Functional changes have consistently been reported at this time in STZ-diabetes, whereas glial cell changes are thought to occur later. This study will isolate neural and Müller cell driven components in the ERG of control and diabetic rats. Photoreceptor responses will be isolated by using L-2-amino-4-phosphonobutyric acid (APB) and *cis*-pipyridine-dicarboxylic acid (PDA) to block neurotransmission from photoreceptors to ON and OFF-bipolar cells, respectively [19]. The Müller cell contribution to the ERG will be isolated using barium chloride (BaCl_2) [20], a potent blocker of inward rectifying potassium channels [21–23], found on the end feet of Müller cells [24]. It is these potassium channels that are involved in the buffering of extracellular potassium build-up that occurs following light activation. Specifically, the potassium buffering activity that follows the light-induced cessation of the dark-current (the fast-P3 component) is thought to generate the slow-P3 component of the ERG [25]. This pharmacological approach allows us to expose the slow-P3 component which, under normal conditions, occurs with a similar time course to the corneal positive bipolar cell driven P2, such that the interaction between these generators produces the ERG b-wave.

Materials and methods

All experimental methods and animal care procedures conform to the ARVO and NHMRC guidelines for animal care and experimentation, and they were approved by a University of Melbourne Animal Ethics Committee (0708732.1).

Experiments were performed on Long Evans rats 6 weeks of age at the time of diabetes induction. Animals were maintained in a 22°C environment with a normal light cycle (12 h at 40 lux, lights on at 8 a.m.); chow (WEHI, Barastoc, VIC, Australia) and water were available ad libitum. These light

levels are below those previously shown to cause retinal light damage [26]. Cages were rotated periodically from top to bottom shelves to avoid bias in light exposure.

Diabetes induction

Animals were fasted overnight (~12 h) prior to diabetes induction via a tail vein injection of 65-mg/kg streptozotocin (MP Biomedicals, Seven Hills, NSW, Australia) dissolved in 0.1-M sodium citrate buffer (pH 4.5, Sigma-Aldrich, Castle Hill, NSW, Australia). Animals given tail vein injections of 0.1-M sodium citrate buffer served as the control group. One week post-STZ injection, animals were deemed to be diabetic if blood glucose (Ascensia™ Esprit Glucometer, Bayer Australia Ltd., Pymble, NSW, Australia) was greater than 15 mmol/l (diabetic: 29.2 ± 1.2 vs. control: 4.1 ± 0.5 mmol/l). In order to prevent ketoacidosis and excess loss of body weight, 2 units of insulin was administered each day (10–12 p.m.).

Electroretinography

As previous [27], dark-adapted rats (>12 h) were anaesthetized with a mixture of ketamine (60 mg/kg) and xylazine (5 mg/kg; Troy Laboratories Pty Ltd., Smithfield, NSW, Australia) via intramuscular injection, with a maintenance dose of 50% of the initial bolus provided every 50 min. Mydriasis was achieved with a drop of tropicamide (0.5%, Alcon Laboratories, Inc., Fort Worth, TX) and phenylephrine (2.5%, Minims, Chauvin Pharmaceuticals, Surrey, UK) and corneal anaesthesia with proxymetacaine (0.5%, Alcon Laboratories, Inc.). Animals were lightly secured to a water-heated platform (37–38°C) to prevent the confound associated with heat loss [28].

Retinal function was assessed across a wide range of flash energies (-6.79 to $2.07 \log \text{cd.s.m}^{-2}$). Signals were recorded using custom-made chlorided silver electrodes (99.99% purity, 0.329 mm 29 G A&E Metal Merchants, Sydney, NSW, Australia). The reference loop electrode (5 mm diameter) rested behind the limbus, and the active was centred on the cornea. Both were referenced to a stainless steel ground (F-E2-60, Grass Technologies, West Warwick, RI) inserted in the tail. Eyes were lubricated after electrode placement and periodically throughout the session with 1.0% carboxymethylcellulose sodium (Celluvisc, Allergan, Irvine, CA).

Signals were sampled at 4 kHz (Powerlab 8SP, ADInstruments) with a band-pass of 0.3–1,000 Hz (-3 dB , P511 AC Preamplifier, Grass Telefactor, West Warwick, RI). Light stimuli were brief (1 ms) white flashes (5 W LEDs, 5,500 K; Luxeon Calgary, Alberta, Canada) delivered via a Ganzfeld integrating sphere (Photometric Solutions International, Huntingdale, VIC, Australia). Flash energy was calibrated for

the rat eye (IL1700; International Light Research, Peabody, MA), and it will be specified in this manuscript in terms of log scotopic cd.s.m^{-2} .

Pharmacological manipulation

All pharmacological agents were diluted in distilled water (dH_2O) and delivered via a sterile 30 G needle attached to a 10- μl Hamilton syringe (SGE Analytical Science Pty, Ltd., Victoria, Australia), via a polyethylene tubing (0.80×0.40 mm inner diameter; Microtube Extensions, North Rocks, NSW, Australia). The needle was inserted through the sclera at a 45° angle, approximately 1.5 mm posterior to the limbus. A plastic cuff was placed around the needle, leaving 1 mm of the tip exposed, to standardize the depth of needle penetration. A retractable arm held the needle in position during injection and throughout the protocol. This approach was adopted such that multiple drugs could be sequentially injected into the same eye, without the need to re-puncture the eye or reset the ERG electrodes.

To allow for sequential drug injection, the tubing was first primed with saline. Small air bubbles (~ 0.50 μl) separated each drug bolus (2.0 μl per agent), as well as the saline column, to prevent mixing. Each injection occurred over 10 s to avoid intraocular pressure elevation. The concentrations for all pharmacological agents represent the calculated final vitreal concentration, based on complete dilution in a rat vitreal volume of 40 μl (Dureau et al., 2001) and no leakage.

Experimental protocol

Müller cell associated K^+ buffering was inhibited by intravitreal injections of 2.0 μl of BaCl_2 (2 mM) into a randomly assigned eye. The contralateral ‘control’ eye of each animal was injected with 2.0 μl of dH_2O . To ensure a stable drug effect, ERG responses to a single luminous energy (at -1.42 log cd.s.m^{-2}) was measured prior to injection and tracked every 5 min post-injection for 40 min. Once effects had stabilized (usually 30–40 min), a scotopic ERG series was measured (-6.79 to 2.07 log cd.s.m^{-2}). After the completion of the first series, a combination of APB (2 μl , 1 mM in dH_2O) and PDA (2.0 μl , 5 mM in dH_2O) was injected into both the BaCl_2 and control eyes to inhibit all post-receptoral contributions to the ERG. The eye that already had BaCl_2 would then lack a Müller cell driven slow-P3 component. Drug effects were tracked for 30 min, and then, a full scotopic ERG series was measured.

Electroretinogram data analysis

Waveforms were analysed by evaluating the amplitude at fixed times of 8, 40 and 110 ms after stimulus onset. Other analysis specific to ERG components is detailed next.

Photoreceptor response

The leading edge of the rat a-wave reflects photoreceptor activity [29, 30]. This was quantified by modelling the first 6 ms over an ensemble of the brightest luminous energies (1.50 and 2.07 log cd.s.m^{-2}) using a delayed Gaussian function [31, 32]. The photoresponse, as a function of time and luminous energy, is given by a saturated amplitude (R_{mP_3} ; μV), sensitivity (S ; $\text{m}^2.\text{cd}^{-1}.\text{s}^{-3}$) and a delay (t_d ; ms fixed to the average of 4.07 ms for normal rats and 4.15 ms for diabetic rats). Parameter optimization was achieved by minimizing the sum-of-squares merit function (Excel™ SOLVER; Microsoft®, Redmond, WA).

ON-bipolar cell response

The most prominent component of the ERG, the corneal positive b-wave, is dominated by the activity of the ON-bipolar cells [33] and is exposed by digitally subtracting the modelled fast-P3 from the raw ERG to expose the P2-OP (oscillatory potential) complex. P2 is then returned by low-pass filtering of the P2-OP complex (55 Hz, -3 dB). P2 peak amplitude and peak time were returned.

Scotopic threshold response The scotopic threshold response (STR) in mice and rats has been shown to contain ganglion cell and some amacrine cell contributions [34–36]. STRs were identified over a range of luminous energies (-6.79 to -5.25 log cd.s.m^{-2}), and amplitudes were measured at fixed times of 110 ms (A110) to coincide with positive component of the STR (pSTR).

Oscillatory potentials OPs are thought to reflect inner retinal feedback [37]. They were isolated by first subtracting the a-wave and then band-pass filtering (5th order Butterworth, -3 dB at 55 and 210 Hz). The largest peak and associated peak time were extracted.

Statistics

Group data are expressed as averages ($\pm$ SEM). Comparison between control and diabetic groups is conducted using a two-way ANOVA (condition vs. luminous energy) with normality and sphericity established using D’Agostino–Pearson normality test and Bartlett’s test, respectively (GraphPad Prism v5.01, San Diego, CA). Comparisons between diabetic and control eyes are also carried out after normalizing to the a-wave minimum to allow ERG components to be considered at a fixed photoreceptor output.

Results

Figure 1 shows that at 4 weeks after injection of STZ, ERG responses show subtle changes. In particular, panel a shows that the b-wave appears to be larger and slower in diabetic rats, whereas the a-wave appears unaffected. Panel b shows that at the dimmest light levels, the STR was smaller in diabetic rats. Quantification of the data shows that the saturated phototransduction amplitude (Fig. 1c, $t=0.45$, $p=0.66$) was unaffected, whereas the phototransduction sensitivity was significantly reduced (Fig. 1d, $t=2.19$, $p=0.04$).

Figure 1e shows that the isolated P2 amplitude as a function of luminous energy was significantly ($F_{1,17}=6.92$, $p=0.009$) increased in diabetic eyes, without an interaction effect ($F_{1,23}=0.13$, $p=1.00$). The most prominent difference between diabetic and controls was an average delay of 17.7 ms (at the highest luminous energy) in peak time ($F_{1,17}=75.14$, $p<0.001$), without a significant interaction ($F_{1,23}=0.32$, $p=0.99$). Figure 1g shows that oscillatory potentials were significantly reduced in STZ diabetic eyes, particularly at higher luminous energies ($F_{1,23}=1.90$, $p=0.007$),

which was revealed by post-hoc analysis to be significant at 1.95 and 2.07 log cd.s.m⁻². Figure 1h shows that there was a significant reduction in pSTR amplitude ($F_{1,17}=4.19$, $p=0.04$).

Given the significant reduction in phototransduction sensitivity, we next considered whether this also manifests as a delay in the deactivation of phototransduction. This was achieved by inhibiting all post-receptor responses using APB and PDA, as well as Müller cell slow-P3 using BaCl₂. In order to facilitate this comparison, waveforms normalised to the a-wave minimum are shown in Fig. 2a. At high luminous energies, after the activation of phototransduction, there is an initial relaxation back to approximately half the saturated amplitude; this is followed by a slower phase of recovery to baseline, which takes approximately 350 ms.

It is clear that in comparison to the 95% confidence limits of controls, diabetic responses have similar recovery. Figure 2b confirms that for a fixed time of 8 ms, the diabetic eyes had significantly smaller responses ($F_{1,17}=11.50$, $p<0.001$), consistent with the reduction in sensitivity (Fig. 1d). In addition, Fig. 2c shows that there was a significant interaction ($F_{1,21}=2.51$, $p<0.001$) for the amplitude at a fixed time of

Fig. 1 Comparisons of retinal function between control and diabetic rats. **a** Averaged dark-adapted, full-field electroretinogram waveforms and **b** scotopic threshold responses over a range of luminous energies (−6.79 to 2.07 log cd.s.m⁻²) for control (dark grey trace, $n=8$) and diabetic (black trace, $n=9$) eyes after 4 weeks of streptozotocin (STZ) injection. All waveforms are normalised to each eye's a-wave minimum at 2.07 log cd.s.m⁻². Light grey areas denote the 95% confidence limits for the control

group. **c** Averaged ($\pm$ SEM) saturated amplitude of phototransduction (R_{mP3} , μ V) and **d** sensitivity ($\log(S)$, m².cd⁻¹.s⁻³) for control (unfilled bars) and diabetic (filled bars). **e** P2 amplitude (ON-bipolar cell function), **f** P2 peak time, **g** oscillatory potential amplitude and **h** scotopic threshold response (amplitude at a fixed time of 110 ms) for control (unfilled symbols) and diabetic (filled symbols) animals. Asterisks denotes significant difference (* $P<0.05$; *** $P<0.0001$)

Fig. 2 Comparisons of the corneal negative fast-P3 isolated using BaCl_2 , APB and PDA in control and diabetic animals. **a** Averaged waveforms for control (dark grey trace) and diabetic (black trace) eyes. All waveforms are normalised to each eye's a-wave minimum at 2.07 $\log \text{cd.s.m}^{-2}$. Grey areas denote 95% confidence limits for the control group. **b** Averaged ($\pm$ SEM) amplitudes at fixed times of 8 and 40 ms (dashed reference lines in **a**) for control (unfilled symbols) and diabetic (filled symbols) eyes. Asterisks denotes significant difference (** $P<0.05$; *** $P<0.0001$)

40 ms, whereby, at intermediate luminous energies, the amplitude was larger in diabetic eyes. At higher luminous energies, there was no difference in amplitude.

Next, we considered the Müller cell mediated response in diabetic and control eyes by comparing APB- and PDA-treated waveforms. In this case, the waveforms contain contributions from both fast- and slow-P3 components. As we have already shown that fast-P3 recovery (amplitude at 40 ms) is similar between diabetic and control eyes, any difference in the APB/PDA treated waveforms could be attributable to a reduction in the Müller cell slow-P3.

Figure 3a shows the difference in the APB/PDA-treated eye with and without BaCl_2 . This shows that the BaCl_2 sensitive feature in the APB/PDA isolated response is a slow corneal negative component. Figure 3b shows the response normalised to the photoreceptor output. It is clear

that the secondary trough after the a-wave minimum (BaCl_2 -sensitive component) was smaller in diabetic rats. Figure 3c confirms that there was a significant interaction between diabetic and control eyes ($F_{1,21}=2.05$, $p=0.005$) in the photoreceptor component (amplitude at 8 ms). Figure 3d shows that at 40 ms, there was a significant interaction ($F_{1,21}=2.36$, $p<0.001$), with the diabetic response being smaller at higher luminous energies.

Figure 4 summarizes the effect of diabetes on the various components of the ERG waveform. In order to facilitate comparison between components, each has been first normalized to the a-wave minimum; in this way, the data are expressed for a common photoreceptor output. We reason this to be appropriate as we have already shown that the saturated phototransduction amplitude (difference $+2.6 \pm 5.2\%$) is similar in diabetic and control groups. Thus, after

Fig. 3 Comparisons of fast- and slow-P3 waveforms isolated using APB and PDA between control and diabetic animals. **a** Representative waveforms (μV) of the difference between APB/PDA with and without BaCl_2 at selected luminous energies ($\log \text{cd ms}^{-2}$). **b** Averaged isolated fast/slow-P3 waveforms for control (dark grey trace) and diabetic (black trace) eyes. All waveforms are normalised to each eyes' a-wave elicited

at 2.07 $\log \text{cd.s.m}^{-2}$. Light grey areas denote 95% confidence limits for the control group. Panels **c** and **d** shows averaged ($\pm$ SEM) amplitudes at fixed times of 8 and 40 ms (dashed reference lines in **b**), respectively, for control (unfilled symbols) and diabetic (filled symbols) eyes. Asterisks denote significant difference (** $P<0.05$; *** $P<0.0001$)

Fig. 4 Effect of diabetes on various components of the electroretinogram. Averaged ($\pm$ SEM) relative response (% difference, [(diabetic - control)/control]). **a** Phototransduction amplitude (RmP_3). **b** ON-bipolar cell P2, scotopic threshold response (pSTR, 110 ms), oscillatory potential (OP), fast-P3 (40 ms) and slow-P3 (40 ms) amplitudes. **c** Phototransduction sensitivity ($\log(S)$), P2 and OP peak times. P2 peak, OP peak and fast- and slow-P3 amplitudes are an average of response elicited at the top

three luminous energies (1.50, 1.95 and 2.07 $\log \text{cd.s.m}^{-2}$). For the pSTR, amplitudes were averaged across -5.94 , -5.52 and $-5.25 \log \text{cd.s.m}^{-2}$. Asterisks denote significance. Dashed reference line denotes no change from controls, 0%. *PRa* photoreceptor activation, *PRd* photoreceptor deactivation, *BC* ON-bipolar cell, *RGC* retinal ganglion cells, *AC* amacrine cell, *MC* Müller cell

normalisation to the a-wave, differences due to diabetes ((diabetic - averaged control)/averaged control,%) between post-receptor ERG components can be compared. When expressed in this way, the P2 was $11.2 \pm 8.6\%$ larger and $22.7 \pm 6.7\%$ slower in diabetic eyes. Despite this P2 amplitude increase, oscillatory potentials were $25.3 \pm 6.0\%$ smaller and $25.8 \pm 9.4\%$ slower. In addition, the STR was $32.7 \pm 9.8\%$ smaller in diabetic eyes, which was similar to the attenuation in the oscillatory potentials ($t=0.65$, $p=0.52$). Our analysis shows that at 40 ms following stimulus onset, the deactivation of the fast-P3 was not different ($-0.1 \pm 2.8\%$) between diabetic and controls, whereas the slow-P3 was $15.7 \pm 6.8\%$ smaller in diabetic eyes, which was similar to the amount of STR loss ($t=1.34$, $p=0.20$).

Discussion

In this study, we considered whether STZ-induced diabetes produces dysfunction in neuronal and glial components of the ERG using pharmacological isolation of components. One difference between ours and previous studies of STZ-induced diabetes is that we opt to normalize post-receptor amplitudes to the photoreceptor driven output, an approach often used when evaluating serial waveforms of an ERG [38]. Thus, we show after 4 weeks of hyperglycaemia induced by STZ-injection that ERG components reflecting the activity of inner retinal neurons, including the STR and the oscillatory potentials, are most affected. This is consistent with previous studies that have revealed greater inner retinal deficits in oscillatory potentials [14] and STR [16, 27] than other features of the ERG. The STR in rats is believed to be largely dependent on the spiking activity of

ganglion cells and to a smaller extent on spiking and graded responses from amacrine cells [34]. Oscillatory potentials are also thought to rely on circuits in the proximal retina involving amacrine cells [37].

We extend previous studies by using pharmacological agents to better expose components of the fast and slow corneal negative components of the ERG. We employ APB and PDA, a combination of agents that are well-characterized inhibitors of all post-receptor responses [19, 39], to expose the photoreceptor and Müller cell mediated buffering of the outer retinal potassium source. In addition, we use BaCl_2 , a non-specific inhibitor of inward rectifying potassium channels, which has been shown to remove the slow-P3 [40]. We show that despite a significant reduction in the sensitivity of the phototransduction cascade, the recovery of the fast-P3 was similar in diabetic rats (Figs. 2 and 4). This outcome suggests that deactivation of phototransduction is unaffected at 4 weeks following hyperglycaemia induction. This is consistent with the finding of Phipps et al. who showed using a twin flash approach that the deactivation of phototransduction was unaffected at 12 weeks of STZ-diabetes [41]. The reduction in phototransduction sensitivity may reflect inefficiencies in the elements of the phototransduction cascade. Kowluru et al. [42] have found that at 2 weeks of STZ-diabetes, there was a significant reduction in the expression of transducin in diabetic rod outer segments.

By assessing the APB/PDA waveform in the absence of BaCl_2 we could show that when normalized for photoreceptor output, the Müller cell derived slow-P3 is smaller in diabetic eyes. This outcome suggests that at 4 weeks after the induction of STZ-diabetes, Müller cells are dysfunctional. This corneal negative component (slow-P3), which is sensitive to BaCl_2 , has been shown to be absent in mice

lacking inward rectifying potassium channels (subtype Kir4.1 [40]), normally found on Müller cell end feet [40, 43]. It is worth noting that impaired potassium siphoning across the retinal pigment epithelium apical membrane may also influence the slow-P3 [44]. This is pertinent as retinal pigment epithelium derived responses have been shown to be altered in STZ-diabetic rats [17]. We feel that the concurrent inner retinal changes found here and the normal dark-adaptation reported by Phipps et al. [41] in diabetic rats argue against a purely retinal pigment epithelium driven deficit. Indeed, our finding that Müller cell dysfunction is concurrent with a reduced STR may not be surprising as inward rectifying potassium channels, specifically Kir4.1 and Kir2.1, have also been associated with generation of the STR [43]. Thus, an overall glial cell dysfunction may

well account for much of the slow-P3 and inner retinal dysfunction observed in our study.

Bringmann and co-workers [45] have found a down regulation of potassium conductance in Müller cells isolated from subjects with proliferative diabetic retinopathy. Consistent with this study, Pannicke et al. [46] show that 6 months of STZ-diabetes in rats reduce potassium conductance towards the proximal end feet of isolated Müller cells. Recently, Curtis et al. [47] show that 4 months after the induction of STZ-diabetes, the localization of Kir4.1 in the inner limiting membrane on Müller cell end feet associated with blood vessels was reduced. These authors also reported that reduced potassium channel localization was associated with accumulation of advanced glycation end-products. These data are consistent with our interpretation that impaired

Fig. 5 Modelling functional changes in diabetic rat electroretinogram waveforms. **a** Upper panel subtraction of the dH₂O/APB/PDA (fast-/slow-P3, black trace) waveform from the raw ERG (dashed green trace) returns the putative ON-bipolar cell P2 (solid green trace). Lower panel subtraction of the BaCl₂/APB/PDA (green trace) from the dH₂O/APB/PDA (black trace) reveals the slow-P3 (dashed green trace). These isolated control components are modified by the measured percentage difference (see Fig. 4) between control and diabetic eyes. **b** Upper panel the control P2 (green trace) is delayed by 22.7%

(blue trace). Lower panel the control slow-P3 (dashed green trace) is reduced by 15.7% and delayed by 22.7% (blue trace). These modified components are added to the fast-P3 recorded from diabetic eyes (red trace). **c** Averaged normalised (to the a-wave minimum) dark-adapted waveforms for control (green dashed traces) and diabetic (red traces) eyes. Composite model waveforms (blue traces) closely resemble the measured diabetic waveforms. **d–h** Average ($\pm$ 95% confidence limits) of the residuals between the model and the diabetic waveform (blue) and the control and the diabetic waveforms (green)

potassium buffering produces a reduction in the slow-P3 at 4 weeks of diabetes.

It is of interest that we also found that the ON-bipolar cell derived P2 was larger and slower. The timing delay can be attributed to the reduction in phototransduction sensitivity, but the amplitude increase cannot. Figure 5 considers how this might arise. Here, we use the pharmacologically isolated components from the control group to model the observed change in the diabetic waveform. Figure 5a shows that subtraction of APB/PDA isolated waveform from the raw ERG returns the putative ON-bipolar cell P2. Also, in the lower panel of Fig. 5, subtraction of the BaCl₂/APB/PDA from the APB/PDA waveform returns the Müller cell derived slow-P3. We then modified these components by the average change found between diabetic and control eyes (Fig. 4). Figure 5b shows that the control P2 is delayed by 22.7% and the slow-P3 is reduced by 15.7% and delayed by 22.7%. These model components are then added to the average fast-P3 from diabetic eyes (Fig. 3). Figure 5c shows that the composite model waveform closely matches the actual response recorded from diabetic eyes. Figure 5d–h shows that the residuals of the model (model–diabetic waveform) encompass zero, whereas the residuals calculated from the controls (control–diabetic waveform) show distinct departures from zero at early and later times. Based on this modelling, we believe that a delay in ON-bipolar cell P2 and slow-P3, as well as a reduction in the slow-P3, is adequate to account for the b-wave changes seen in our diabetic eye. The P2 and slow-P3 delay is likely to reflect the reduction in sensitivity of the phototransduction cascade.

Summary

We show that after 4 weeks of STZ-diabetes, there were a delay in the phototransduction cascade and reductions in the STR and oscillatory potentials, as well as the BaCl₂ sensitive slow-P3. We interpreted the slow-P3 loss to indicate that Müller cells are dysfunctional due to abnormalities in inward rectifying potassium channels. We show that glial anomalies occur concurrently with neuronal dysfunction in the STZ-diabetes model.

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