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Research article

Development of diabetes-induced acidosis in the rat retina

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ABSTRACT

We hypothesized that the retina of diabetic animals would be unusually acidic due to increased glycolytic metabolism. Acidosis in tumors and isolated retina has been shown to lead to increased VEGF. To test the hypothesis we have measured the transretinal distribution of extracellular H^+ concentration (H^+-profiles) in retinae of control and diabetic dark-adapted intact Long-Evans rats with ion-selective electrodes. Diabetes was induced by intraperitoneal injection of streptozotocin. Intact rat retinae are normally more acidic than blood with a peak of $[H^+]_o$ in the outer nuclear layer (ONL) that averages 30 nM higher than H^+ in the choroid. Profiles in diabetic animals were similar in shape, but diabetic retinae began to be considerably more acidic after 5 weeks of diabetes. In retinae of 1–3 month diabetics the difference between the ONL and choroid was almost twice as great as in controls. At later times, up to 6 months, some diabetics still demonstrated abnormally high levels of $[H^+]_o$, but others were even less acidic than controls, so that the average level of acidosis was not different. Greater variability in H^+-profiles (both between animals and between profiles recorded in one animal) distinguished the diabetic retinae from controls. Within animals, this variability was not random, but exhibited regions of higher and lower H^+. We conclude that retinal acidosis begins to develop at an early stage of diabetes (1–3 months) in rats. However, it does not progress, and the acidity of diabetic rat retina was diminished at later stages (3–6 months). Also the diabetes-induced acidosis has a strongly expressed local character. As result, the diabetic retinas show much wider variability in $[H^+]$ distribution than controls. pH influences metabolic and neural processes, and these results suggest that local acidosis could play a role in the pathogenesis of diabetic retinopathy.

1. Introduction

While there has been a great deal of work and speculation about changes in oxygen in the diabetic retina, very little is known about another important metabolic parameter, pH. The possibility that acidosis contributes to some of the pathogenesis of diabetic retinopathy is supported by several lines of evidence. First, VEGF, which is acknowledged to be an important player in diabetic retinopathy (Stitt et al., 2015), is known to be upregulated by acidosis independently of hypoxia in glioblastoma (Xu et al., 2002) and pancreatic adenocarcinoma (Fukumura et al., 2001; Shi et al., 2001). VEGF also increases when the isolated rat retina is subjected to acidosis (Zhu et al., 2009). Second, in neonatal retina acidosis can mimic the effect of oxygen-induced retinopathy in causing neovascularization (Holmes et al., 1998; 1999; Leske et al., 2004). Third, acute hyperglycemia markedly acidified the normal cat retina (Padnick-Silver and Linsenmeier, 2005), particularly the inner retina. Thus, we can hypothesize that the hyperglycemia in diabetes increases anaerobic glycolysis and the consequent production of lactate and H^+, and that this could contribute to an increase VEGF, causing at least part of the neovascularization in diabetes, not to mention the many other changes that could occur in retinal function due to acidosis. The only measurements of intraretinal pH in diabetic animals, however, were those made on a very small number of diabetic cats, most of which had long-standing diabetes (7.7–9.4 years) with considerable capillary dropout (Budzynski et al., 2005). The findings in those animals were puzzling. In a normal animal, the point of highest $[H^+]_o$ in the retina is in the

Abbreviations: STZ, streptozotocin; ERG, electroretinogram; $[H^+]_o$, extracellular hydrogen concentration.

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outer nuclear layer, with decreasing H^+ toward the choroid and toward the vitreous. In many profiles in the long-term diabetics, the inner retina was the most acidic part of the retina, and the outer retina did not exhibit the usual high value of H^+. We hypothesized that failure of photoreceptor glycolysis had also occurred, which reduced H^+ production, as the photoreceptors are affected in diabetes (Kern and Berkowitz, 2015; Scarinci et al., 2015). In one cat with only 2.1 years of diabetes the shape of the H^+ profiles was more normal, and the major change was acidification (Budzynski et al., 2005), as we had originally expected. However, the fact remains that there have been very limited measurements. It has not been possible previously to study how retinal pH changes over time in diabetic animals, and how $[H^+]_o$ gradients are altered as the disease progresses. The only other work to date on retinal H^+ in diabetes has been our recent study on changes in intraretinal $[H^+]_o$ evoked by flashes of light (Dmitriev et al., 2016). Light-evoked responses were altered only distal to the RPE in the choroid, suggesting possible dysfunction in the ability of the choroid to clear H^+ from the retina during diabetes.

The present study was therefore undertaken to close the gap in basic knowledge about retinal pH in diabetes. Because it is difficult to study diabetes in a large animal model, we measured intraretinal profiles of $[H^+]_o$ *in vivo* with H^+-sensitive microelectrodes in diabetic rats at different time points up to 27 weeks after the induction of diabetes, and compared the findings to those in age-matched control animals. Our hypothesis was that the diabetics would be more acidic than controls during at least some period of time, although possibly not continuously, based on what had been observed in cat (Budzynski et al., 2005). Further, because of the work on acute hyperglycemia in cats, we expected especially the inner retina to be more acidic than normal. These hypotheses were partly supported by the data.

2. Material and methods

2.1. Animal preparation

Animal experiments were performed in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by Northwestern University's Institutional Animal Care and Use Committee. Adult male Long-Evans rats were anesthetized with 2.5–3% isoflurane/35% O_2 during preparation, and anesthesia was gradually switched to urethane (800 mg/kg loading followed by 75 mg-kg^{-1}-h^{r-1}) supplemented with 0.5% isoflurane. After completing surgical preparations, the animal was paralyzed with pancuronium bromide and artificially ventilated. An arterial cannula was used to measure blood pressure and to take blood samples for measurements of PaO_2, $PaCO_2$, pHa and glucose. The tidal volume provided by the respirator and the fraction of inspired oxygen were adjusted to maintain arterial values in the normal range of $P_aO_2 \approx 100$ mmHg, $P_aCO_2 \approx 40$ mmHg and pHa ≈ 7.4. The animal's body temperature was measured with a rectal probe and used to control a water filled heating pad to maintain temperature at 37° C. Needle leads were used to measure heart rate from the ECG. Further details of the preparation have been given previously (Lau and Linsenmeier, 2012). Forty-one rats (18 controls and 23 diabetics) were studied. Light-evoked H^+ responses have been reported from some of these same animals (Dmitriev et al., 2016).

2.2. Induction of diabetes

Diabetes was induced with a single intraperitoneal injection of STZ and age-matched controls received a single intraperitoneal injection of 0.05 mol/L sodium citrate buffer only as reported previously (Dmitriev et al., 2016). Rats were weighed weekly, and nonfasting blood glucose levels were measured from the tail vein using a Bayer CONTOUR Meter (Bayer HealthCare LLC, Mishawaka, IN). In diabetics, average blood glucose was 519 ± 74 mg/dL (mean ± s.d.) during weekly measurements prior to the H^+ recordings. During the experiments, the blood glucose was usually lower (in 22 out of 23 cases) and the average was 407 ± 80 mg/dL, which was still much higher than in controls (137 ± 41, n = 17). Data presented here were obtained from animals after 2–27 weeks (0.5–6 months) of diabetes.

2.3. $[H^+]_o$ recordings

Double-barreled H^+-sensitive microelectrodes used to record intraretinal $[H^+]_o$ were constructed using methods described previously for Ca^{2+}-sensitive microelectrodes (Dmitriev et al., 1999), except that the electrodes were filled with Hydrogen Ionophore I – Cocktail A or Cocktail B (Fluka). One of the barrels served to record voltage and the other contained an H^+-sensitive liquid and therefore recorded the H^+ equilibrium potential across the liquid plus voltage. Both ion-selective and reference barrels were backfilled with a simplified Ringer's solution (140 mM NaCl, 5 mM KCl, 10 mM HEPES, pH = 7.4). After filling, the tip of the microelectrode was beveled on a micropipette beveler (BV-10, Sutter Instrument Co.) so that the tip diameter was 5–8 μm. The resistance of the H^+-selective barrel was 50–70 GΩ; the reference barrel had a resistance of 60–100 MΩ. Electrodes were calibrated in solutions that were similar to the standard Ringer's and had pH values of 7.0, 7.4 and 7.8. The slope of the H^+ electrodes was close to Nernstian. Voltage from the H^+ barrel was recorded with a high input impedance amplifier (FD-223a, WPI).

The microelectrode was placed inside a metal needle inserted into the eye through the sclera and advanced through the vitreous toward the retina as described earlier (Lau and Linsenmeier, 2012). The rat's head was adjusted so that the cornea of the right eye faced upward, and the visual axis was oriented vertically. The microelectrode penetrated the sclera on the superior side of the eye near the equator at an angle of 32° or 34° and hit the retina about 1 mm inferior to the disc. To obtain a series of H^+-profiles, the electrode was adjusted in successive penetrations by rotating it about the entry point on the eye in steps of 2° so that the penetrations covered up to 30° in the nasal to caudal direction. Both the voltage and ion-selective barrels were referenced to an Ag/AgCl electrode in the neck. When in the retina, as signaled by a change in the electroretinogram, the electrode was advanced in 30 μm steps by a hydraulic micropositioner (Kopf model 2650) until penetration of the retinal pigment epithelium. From there the electrode was continuously withdrawn at 1 μm/sec back to the vitreous. The choroid/retinal boundary was considered to be the point at which $[H^+]$ began to increase. During withdrawal, short dim flashes (white light, 7.5 lux for 100 msec) were delivered at 10 s intervals to record the intraretinal ERG. The vitreoretinal interface was identified as the location where the intraretinal ERG recorded by the voltage barrel of the microelectrode during withdrawal was the same as the vitreal ERG. To obtain the pure H^+-dependent voltages, the voltages recorded by the voltage barrel of the electrode during the withdrawal were subtracted from the simultaneously recorded voltages from the H^+-selective barrel. The resulting H^+-selective voltages (Fig. 1A) were recalculated into H^+-profiles in the following 3 steps. First, the voltage of the H^+-selective microelectrode (in mV) was converted to $[H^+]$ (in nM) based on a calibration of each electrode performed before the experiment. Absolute values of extracellular H^+ concentration ($[H^+]_o$) were obtained by assuming that $[H^+]$ in the choroid during each penetration was the same as arterial $[H^+]$ measured just before or after each profile.