Effect of Hypoxemia and Hyperglycemia on pH in the Intact Cat Retina

Lissa Padnick-Silver, PhD; Robert A. Linsenmeier, PhD

Objective: To examine the effects of acute hypoxemia and hyperglycemia on retinal pH to understand hyperglycemia-induced changes in the normal intact cat retina.

Methods: Spatial profiles of extracellular hydrogen ion (H+/H11001) concentration were obtained from the cat retina, in vivo, using pH-sensitive microelectrodes during normoxia (arterial partial pressure of oxygen [PaO2] = 114.5 ± 7.9 mm Hg), normoglycemia (plasma glucose concentration, 117 ± 19 mg/dL), acute hypoxemia (PaO2 = 29.5 ± 2.2 mm Hg), and acute hyperglycemia (plasma glucose concentration, 303 ± 67 mg/dL). An H+/H11001 diffusion model was fitted to the outer retinal data to quantify photoreceptor H+ production. The inner retinal pH was also examined.

Results: Hypoxemia induced a mean acute panretinal acidification of 0.16 pH units that originated from a 2.55-fold increase in net photoreceptor H+ production. Hyperglycemia induced an acute panretinal acidification of 0.12 pH units; however, photoreceptor H+ production levels remained unchanged. Retinal pH changes followed the course of arterial PaO2 and blood glucose changes.

Conclusions: The increase in photoreceptor H+ production during hypoxemia confirms the importance of glycolysis in the retina. Hyperglycemia-induced pH changes resulted from either increased inner retinal H+ production or decreased H+ clearance/neutralization.

Clinical Relevance: The hyperglycemia-induced acidification that originates in the inner retina suggests that retinal acidosis may contribute to the development of diabetic retinal disease.


Understanding acute changes resulting from hyperglycemia in a normal retina may aid in the discovery of mechanisms involved in the development and progression of diabetic retinopathy. Oxygen studies have shown decreases in retinal oxygenation in diabetic animals with little to no diabetic retinal disease (as assessed by direct ophthalmoscopy). These changes were not observed during acute hyperglycemia in healthy animals. The retina does not rely solely on oxidative metabolism, however; it is also heavily dependent on glycolytic metabolism. Because the use of each glycolytically produced adenosine triphosphate (ATP), but not the use of each oxidatively produced ATP, results in a net gain of 1 hydrogen ion (H+), we expected the retina to acidify with an increase in retinal glycolysis. In this study, we examined hyperglycemia-induced changes in retinal pH and photoreceptor glycolysis to determine their involvement in diabetic retinopathy.

It was recently shown that the retina exists in a delicate H+ production/elimination balance. To prevent acidosis, the tissue must possess extremely efficient H+ clearance/neutralization mechanisms, possibly involving retinal pigment epithelial and/or Müller cell active H+ and/or bicarbonate ion (HCO3-) transport.

Hypoxemia, a condition known to increase outer retinal glycolysis, was examined to study retinal pH during hypoxemia and to aid in the interpretation of changes during hyperglycemia. Decreases in partial pressure of oxygen (PaO2) do not always cause decreases in retinal tissue oxygen levels, but for the severe hypoxemia evaluated in our study, both the outer and inner retina were expected to be hypoxic.

METHODS

EXPERIMENTAL PREPARATION

All efforts to minimize animals’ pain and discomfort and all procedures followed the guidelines
of the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Laboratory Research. Eight adult male cats were anesthetized with sodium pentothal (loading dose: 22 mg/kg intravenously, and as needed during surgery). Urethane was also given (400 mg/h) during surgery until a loading dose of 300 to 400 mg/kg was reached. A complete experimental description was published previously.7

All recordings were made while the animal was paralyzed and receiving urethane anesthesia. The animal was artificially ventilated at a rate and volume suitable for maintaining arterial blood gas values within a normal range (ie, PaO2>90 mm Hg; PaCO2<30 mm Hg; pH 7.35<pH<7.45). Blood glucose was measured every 30 to 45 minutes and was maintained between 80 and 120 mg/dL (4.44-6.66 mmol/L) through continuous intravenous infusion of glucose (40 g/100 mL) and/or regular porcine insulin (500 mU/mL; Iletin, Eli Lilly, Indianapolis, Ind) during normoglycemic recordings. All electrode recordings were made in the area centralis, the region of maximum acuity in the cat retina, during dark adaptation. Additionally, 1 intravitreal 20-gauge needle accommodated a silver/silver chloride voltage electrode for electroretinographic recording. This type of electrode was also sutured into the scalp to provide a common reference for all electrodes.

Following stepwise penetration of the retina with the electrode, the pH was continuously recorded during electrode withdrawal from the choroid to the vitreous at 1 µm/s, generating a pH “profile” across the retina. The pH data were digitized at 20 Hz and successive groups of 10 values were averaged, effectively reducing the data acquisition frequency to 2 Hz.

**pH MICROELECTRODES**

Double-barreled pH microelectrodes recorded the intraretinal pH, which was later converted into extracellular H+ concentration ([H+]o). The complete fabrication process has been described previously.7 Electrodes were calibrated in buffered Ringer solutions of pH 6.0, 7.0, and 8.0 to obtain mean±SD electrode sensitivity, which was 48.7±7.0 mV/pH unit (n=8 electrodes).

The pH electrodes do not measure absolute pH, so a known value of pH must be used as a reference. It was assumed that choroidal pH was the same as arterial pH because the choroidal blood flow rate is very high.11,12 Arterial pH was measured immediately after each profile was recorded.

**INDUCTION OF HYPOXEMIA AND HYPERGLYCEMIA**

Hypoxemia was induced in 5 cats by decreasing the fraction of inspired oxygen (FiO2) from 0.21 (room air) to 0.10. This reduced the mean ± SD PaO2 from 114.5±7.9 mm Hg during normoxia to 29.5±2.2 mm Hg during hypoxemia.

Hypoglycemia (blood glucose >200 mg/dL (>11.1 mmol/L)) was induced by increasing glucose and decreasing insulin infusion rates. Acute hypoglycemia was induced in 6 cats and lasted a mean ± SD duration of 1.9±1.3 hours. This increased plasma glucose levels from 117±19 mg/dL (6.5±1.05 mmol/L) during normoglycemia to 303±67 mg/dL (16.8±3.7 mmol/L) during hyperglycemia. Acute hypoglycemia was examined first in 2 of the 6 cats. Following hypoglycemia, animals were allowed to recover for at least 1 hour before normoglycemia (control) measurements were collected. The cat was considered to be in recovery once the plasma glucose concentration fell below 200 mg/dL (<11.1 mmol/L). The time following hyperglycemia therefore varied between cats but was generally about 30 minutes. In 1 cat the recovery period was longer, approximately 2 hours.

**DATA ANALYSIS**

All values are reported as mean±SD. Statistical significance was determined with unpaired t tests, comparing the averaged results from normoxic and hypoxic values or normoglycemic and hyperglycemic values. Unpaired tests were used because the number of profiles from each cat was small and roughly uniform. Significance was defined as P<.05. Data values that were more than 2 SDs outside the mean results were considered outliers and were removed from the analyses (1 data point from 1 cat).

**NET H+ PRODUCTION**

A steady state mathematical diffusion model for ions moving through extracellular space has been described previously.13 It was modified and validated7 and used in this study to solve for outer retinal H+ production (Q[OH−,H+]). The model has 3 layers (i=1 to 3) corresponding to the anatomical regions of the photoreceptor (outer segment, inner segment, and outer nuclear layer [ONL]). The Q[OH−,H+] in the outer segment layer is assumed to be zero, and it is allowed to be nonzero in the inner segment and ONLs.7

\[
\frac{D}{\lambda_i^2} \frac{\partial^2 C_i}{\partial x^2} + \frac{Q_i}{\alpha_i} = 0
\]

where C, is [H+], in each layer, Q, (moles per liter per second) is net H+ production in each layer (Q=0), λ, is H+ pathway tortuosity (λ2=1; λ3=1.53), α, is extracellular volume fraction (αi=0.1; α3=0.21), and D is H+ diffusivity through plasma at 37°C (D=1.107×10−4 cm²/s). Figure 1 shows a typical [H+]o profile with the modeling results superimposed. All profiles are shown as [H+]o because this is the linear quantity that diffusion laws apply to. The pH profiles were recorded extracellularly; therefore, the H+ production parameter, Q, represents net H+ extrusion into the subretinal space. All reported photoreceptor H+ production values, Q[OH−,H+], are the weighted average across the 3 layers of the outer retina. As in previous work,7,10 values have also been adjusted to account for distortion of the profile during electrode withdrawal.

**RESULTS**

**EFFECTS OF HYPOXEMIA ON RETINAL PH**

Figure 2A shows examples of [H+]o profiles across the dark-adapted cat retina during normoxia and hypoxemia. Under normoxic conditions, the vitreous was slightly more acidic than the choroid, with the most acidic point in the proximal ONL.7,14 Reducing FiO2 from 21% (PaO2=114.5±7.9 mm Hg; n=5 cats) to 10% (PaO2=29.5±2.2 mm Hg) resulted in an acidification of the entire retina. Profile magnitude, the difference between peak [H+]o and choroidal [H+]o, increased from 2.64±0.74×10−8 mol/L during normoxia (n=15 profiles; 5 cats) to 6.09±2.24×10−8 mol/L during hypoxia (n=8 profiles; 5 cats), corresponding to an average acidification of 0.125 pH units. This is consistent with previous measurements in which the maximal acidification was 0.08 pH units in the ONL (PaO2~40 mm Hg).8
The outer retinal tissue acidification resulted from an increase in net H⁺ production (Q_{OR-H⁺}) during hypoxemia. The Q_{OR-H⁺} increased by a factor of 2.55, from $5.22 \pm 2.87 \times 10^{-9}$ mol/L·s during normoxia (n=15 profiles; 5 cats) to $13.32 \pm 8.01 \times 10^{-9}$ mol/L·s during hypoxemia (FIO₂=10%; n=8 profiles; 5 cats; $P=0.02$). Simulated outer retinal [H⁺]₀ distributions, based on average fitted parameter values during normoxia and hypoxemia, show the average effect of hypoxemia on retinal [H⁺]₀ (Figure 2B).

The inner retina also acidified during hypoxemia (Figure 2A). The average inner retinal pH decreased from $7.24 \pm 0.04$ (n=13 profiles; 5 cats) during normoxia to $7.08 \pm 0.10$ (n=8 profiles; 5 cats; $P=0.006$) during hypoxemia.
EFFECTS OF ACUTE HYPERGLYCEMIA ON RETINAL pH

Intraretinal $[H^+]_o$ profiles before, during, and after hyperglycemia are shown in Figure 3A. In contrast to normoglycemic and hypoxic profiles, in which the maximum $[H^+]_o$ was in the ONL, the peak value of $[H^+]_o$ was located in the inner retina during hyperglycemia.

Increasing plasma glucose levels from 117±19 mg/dL (6.5±1.05 mmol/L) to 303±67 mg/dL (16.8±3.7 mmol/L; plasma glucose concentration >200 mg/dL [>11.1 mmol/L]; 2.0±1.3 hours) resulted in an acidification of the entire retina. The pH changes followed the magnitude and course of plasma glucose changes in 5 of 6 cats with virtually no lag (Figure 4). The magnitude of intraretinal $[H^+]_o$ profiles was significantly correlated with blood glucose levels ($r^2=0.45$; $n=72$ profiles from 5 cats; $P=.001$; Figure 4B). In the sixth cat an acidification was observed, but the onset was delayed until glucose levels were decreased. Data from this cat were therefore not included in regression analyses.

The blood pH, and therefore choroidal pH, did not significantly change when the animal was made hyperglycemic. However, the profile magnitude significantly increased from $2.76±0.86 \times 10^{-8}$ mol/L (minimum pH=7.15; $n=10$ profiles; 5 cats) to $4.46±1.96 \times 10^{-8}$ mol/L during hyperglycemia (minimum pH=7.07; $n=10$ profiles; 5 cats) ($P=.01$).

Outer Retina

Net photoreceptor $H^+$ production ($Q_{OR-H^+}$) was $3.15±2.42 \times 10^{-6}$ mol/L·s during normoglycemia (plasma glucose concentration=118±21 mg/dL [6.5±1.2 mmol/L]; $n=10$ profiles; 5 cats) and $3.43±1.69 \times 10^{-6}$ mol/L·s during hyperglycemia (plasma glucose concentration=266±49 mg/dL [14.7±2.7 mmol/L]; $n=10$ profiles; 5 cats), an insignificant difference ($P=.84$). Figure 3A shows example profiles. Figure 3B shows $[H^+]_o$ profile simulations, based on averaged fitted parameters illustrating the average effect of hyperglycemia on the outer retina and emphasizing the reversal of the $H^+$ gradient in the proximal part of the ONL during hyperglycemia. Photoreceptor $H^+$ production during recovery (plasma glucose=97±30 mg/dL [5.3±1.6 mmol/L]) was also not statistically different from that during normoglycemia. Recovery data were generally collected within the first 30 minutes of the recovery phase. In 1 cat, recovery was longer.

Inner Retina

Hyperglycemia (plasma glucose concentration=275±57 mg/dL [15.2±3.1 mmol/L]) acidified the inner retina by an average of $0.12±0.06$ pH units in 5 cats (normoglycemic pH=7.20±0.04; hyperglycemia pH=7.08±0.09; $P=.01$). Returning glucose to normal (plasma glucose concentration=108±28 mg/dL [6.0±1.6 mmol/L]) returned the inner retinal pH to 7.16±0.06, which was not significantly different from the baseline inner retinal pH ($n=3$ cats; $P=.37$).

EFFECTS OF HYPOXEMIA AND HYPERGLYCEMIA ON THE ELECTRORETINOGRAM

No significant effects of hypoxemia on the vitreal scotopic electroretinogram were observed. The b-wave tended to be smaller during hypoxia, whereas the c-wave tended to be slightly larger ($P=.22$ and .58, respectively).
Hyperglycemia decreased the scotopic b-wave by 26.4% ± 16.4% (n = 5 cats; P = .02) and increased the scotopic c-wave by 12.6% ± 8.9% (n = 5 cats; P = .03). These changes are consistent with those caused by systemic acidosis.15,16

**COMMENT**

**EFFECT OF HYPOXEMIA ON RETINAL pH**

**Outer Retina**

An increase in photoreceptor H⁺ production was the source of the hypoxemia-induced retinal acidification. This almost certainly reflects an increase in outer retinal glycolysis to compensate for the hypoxemia-induced decrease in oxidative metabolism.5,9,10,17-20

The observed Q_{OR-H⁺} provides a value for net outer retinal H⁺ production. Our electrodes measure extracellular H⁺ concentration and therefore do not account for H⁺ produced that never exits the cell or is rapidly neutralized by buffers intracellularly or extracellularly. An increase in actual H⁺ production or a decrease in H⁺ uptake could result in an increased net H⁺ production (Q_{OR-H⁺}) during hypoxemia. Experimental evidence, however, indicates that our measure of net H⁺ production accurately reflects relative changes of actual H⁺ production in different conditions.7

During normoxia, photoreceptor oxygen consumption (QO₂) is approximately 4.0 mL O₂/100 g·min in the cat,10 with 80% of choroidal glucose metabolized to lactate.21 During hypoxemia (PaO₂ = 30 mm Hg), QO₂ is 1.68 mL O₂/100 g·min.10 Given this information, one can calculate that the glycolytic rate, and therefore the actual H⁺ production rate, would need to increase by a factor of 3.6 to completely compensate for the loss of oxidative ATP production. Experimentally, net H⁺ production (Q_{OR-H⁺}) increased by a factor of 2.55 (PaO₂ of 29.5 ± 2.2 mm Hg), which undoubtedly reflects the expected increase in glycolysis. Several factors could explain why the net change is smaller than the predicted change in actual H⁺ production. The compensation in glycolysis for the loss of oxidative ATP generation during hypoxemia may explain why the photoreceptor is extremely resistant to hypoxia (reflected by a-wave amplitude).22 We know that decreasing blood glucose can reduce the ability of the photoreceptors to compensate for hypoxemia, providing an additional line of support for the importance of glycolysis in the retina.18

**Inner Retina**

The shape of pH profiles during hypoxemia indicated that the observed inner retinal acidification resulted from an influx of H⁺ from the outer retina and probably an increase in inner retinal glycolysis. Unfortunately, inner retinal glycolytic rates could not be determined because the 1-dimensional H⁺ diffusion model cannot accurately describe the 3-dimensional [H⁺]c distribution resulting from the 3-dimensional network of the retinal capillaries.

The hypoxemia-induced acidification indicates that the increased retinal blood flow during hypoxemia21 is not adequate to clear excess H⁺ generated by the retina. This suggests that the retinal circulation H⁺ clearance capabilities may be nearly saturated in basal conditions.

**EFFECTS OF ACUTE HYPERGLYCEMIA ON RETINAL pH**

**Outer Retina**

Acute hyperglycemia does not change photoreceptor oxygen consumption,2,3,5,21 and in this study we found no significant change in H⁺ production. We therefore conclude that acute hyperglycemia does not cause changes in photoreceptor energy metabolism.

Although outer retinal glycolysis did not change during hyperglycemia, a hyperglycemia-induced acidifica-

![Figure 4. Effect of plasma glucose levels on intraretinal hydrogen ion concentration ([H⁺]) profile magnitude. A, Time course of blood glucose and [H⁺], profile magnitude for a typical experiment. B, The relationship between [H⁺], profile magnitude and blood glucose levels was highly significantly (r²=0.45; n=72 profiles from 5 cats; P=.001). Each cat is represented by its own symbol. Values are represented in compressed scientific notation (eg, 8 × 10⁻⁸ is given as 8e–8). To convert glucose to millimoles per liter, multiply by 0.0555.](image)

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tion originated in the inner retina (Figure 3). This was physiologically different than the hypoxemia-induced acidification, which originated in the outer retina (Figure 2) from an increase in photoreceptor glycolysis. During normoglycemia, protons diffuse from the outer retina to the inner retina, presumably to be carried away by the retinal vasculature. During acute hyperglycemia, the proton gradient in the ONL reverses, indicating that inner retinal H+ clearance/neutralization mechanisms are no longer sufficient and excess H+ must be cleared/neutralized by outer retinal mechanisms (ie, retinal pigment epithelium or choroid).

**Inner Retina**

Acute hyperglycemia in a normal, intact cat retina resulted in an acute inner retinal acidification of 0.12±0.06 pH units (Figure 3A). Because we cannot model inner retinal [H+]o, we do not know if the acidification was due to increased H+ production or decreased H+ clearance. Acute hyperglycemia increases retinal blood flow in animals and humans, which should enhance inner retinal H+ clearance. Also, retinal blood flow increases during retinal acidosis in the presence of hyperoxemia, owing to decreases in tissue pH. It is therefore more likely that the hyperglycemia-induced acidosis was due to increased retinal glycolysis than to decreased clearance. However, we cannot rule out the possibility that hyperglycemia impairs the Müller cell H+/HCO3 active transport processes that may be important in retinal pH homeostasis.

**IMPLICATIONS FOR DIABETIC RETINOPATHY**

Oxygen tension (PO2) across the retina has been characterized in the intact cat retina during normal conditions, acute hyperglycemia and chronic hyperglycemia (diabetes). A significant decrease in inner retinal PO2 was observed in long-term diabetic cats with little to no signs of diabetic retinopathy but not in acutely hyperglycemic cats. These observations lead us to believe that tissue oxygen changes may be secondary to other alterations in the retinal microenvironment, perhaps relating to pH.

**Retinal Oxygen Autoregulation**

A loss of hyperoxic oxygen autoregulation during hyperglycemia has been observed in diabetic rats, dogs, and humans and in healthy dogs. Considering the inner retinal acidification observed in our study, we suggest that the apparent loss of oxygen autoregulation does not necessarily indicate failure of oxygen autoregulatory mechanisms during hyperglycemia. Instead, it may be that pH-mediated changes in retinal blood flow blunt the oxygen autoregulatory responses to hyperoxia. Supporting this theory, vessel caliber responses induced by pH changes have been shown to dominate compared with those induced by oxygen changes in the retina.

**Angiogenesis**

Tissue acidosis generally accompanies tissue hypoxia, and it is therefore reasonable to speculate that changes in pH may play a role in the regulation of angiogenic and antiangiogenic substances. In support of this theory, acidosis alone has been shown to cause retinal neovascularization and to up-regulate vascular endothelial growth factor (VEGF) in human brain tumors, human pancreatic adenocarcinoma cells, and human glioblastoma cells. Xu et al also demonstrated that low pH (6.6) activates VEGF transcription and reversibly alters either VEGF or its cellular binding site. These changes led to an increased affinity of VEGF to its binding site in bovine aortic endothelial cells. Repeated episodes of acute retinal acidosis caused by hyperglycemia may cause a slow, progressive up-regulation of VEGF, but this remains to be proved. Still, the inner retina in diabetic cats tends to be acidotic compared with that of healthy cats.

One must be cautious about extrapolating from short-term hyperglycemia to the more complex situation in diabetes. However, in light of the large hyperglycemia-induced inner retinal pH changes observed in this study, the inner retinal acidosis previously observed in diabetic cats, and the observation that acidosis alone can induce retinopathy, we conclude that pH changes may play a role in the development and progression of diabetic retinopathy in humans.

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