Hyperglycemia Impairs Retinal Oxygen Autoregulation in Normal and Diabetic Dogs

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Preretinal oxygen tensions were measured continuously using microelectrodes in four normal dogs and four dogs made diabetic with alloxan. The latter were kept under poor control for 8 months. Glucose administered intravenously in 30 to 90 sec to all the dogs when they were normoglycemic caused an immediate increase in preretinal oxygen tension lasting approximately 10 min. When the dogs were given 100% oxygen to breathe, their preretinal oxygen tensions increased. This increase in preretal oxygen tension was 36.6 ± 8.5 mmHg (±SE) when the animals were normoglycemic and 63.5 ± 9.2 mmHg when the animals were made hyperglycemic. This suggests that hyperglycemia impairs oxygen autoregulation. Moreover, the diabetic dogs appeared to exhibit a larger preretinal oxygen tension increase than normal dogs when both were given 100% oxygen to breathe. The small number of animals studied, however, makes a comparison between the two groups difficult. These data lead one to speculate that in diabetes there may be an impairment of the normal retinal vascular homeostasis. This could play a role in both the development and in the severity of diabetic retinopathy. Invest Ophthalmol Vis Sci 24:985–989

Diabetes is characterized by elevated blood glucose, and treatment with exogenous insulin decreases the level. This, however, leads to repeated fluctuations in blood glucose levels. Acute hyperglycemia has been shown to increase the retinal blood flow in the normal cat1 and cause retinal vasodilatation in normal humans.2 Moreover, increased retinal blood flow may occur in patients with diabetes.3 To investigate this phenomenon, we studied the acute effects of the infusion of glucose on the preretinal oxygen tension in both normal and poorly controlled diabetic dogs.

Materials and Methods. Fourteen female beagles, 1 to 3 years of age, weighing 10 to 20 kg and having ophthalmoscopically normal eyes, were used in this study. Six of the dogs were made diabetic by an intravenous injection of alloxan monohydrate (55 mg/kg) following a 24-hr fast. The animals, housed in metabolism cages throughout the study, were allowed food ad libitum and maintained by subcutaneous injections of insulin (up to 7 U NPH daily) in dosages insufficient to prevent chronic glycosuria and which caused a gradual increase of glycosylated hemoglobin levels in their blood. Glycosylated hemoglobin was measured using chromatography columns available commercially (Isolab, Inc., Akron, OH), and was found to rise from 5.6% ± 0.9% (±SD) before diabetes was induced to 10.6% ± 0.4% at the time retinal oxygen tensions were measured, 31 to 36 weeks later.

Preretinal oxygen tension measurements were successful in four normal and four diabetic dogs. Insulin was withheld from the diabetic dogs for 24 hr preceding the day of the experiment and all dogs were fasted overnight. The animals were anesthetized with sodium pentobarbital (25 mg/kg IV) and tracheotomized. The animals were then placed on a respirator (Model 607, Harvard Apparatus Company, Millis, MA), and paralyzed with tubocurarine chloride (0.1 mg/kg) and gallamine triethiodide (0.5 mg/kg). The arterial blood pressure was monitored from a cannulated femoral artery with a pressure transducer (Model 1280 C, Hewlett-Packard, Waltham, MA). The intraocular pressure was maintained at 15 mmHg by adjusting the height of an infusion bottle connected to an anterior chamber cannula and to a second pressure transducer. The perfusion pressure was defined as the difference between the mean systemic blood pressure measured in the femoral artery and the intraocular pressure. The perfusion pressure was recorded continuously by electronically subtracting the outputs from the two pressure transducers. All the parameters were recorded on a polygraph (Model 7758, Hewlett-Packard). The animal’s temperature was measured with a rectal thermometer and maintained at 39.0°C with a thermoblock (EKEG, Electronics Co. Ltd.). Arterial blood samples were analyzed throughout each experiment for pH, Pco2, Po2, and oxygen saturation. The animal’s pH was maintained at 7.37 ± 0.07 by adjusting the respiratory rate and, with the tidal volumes used, this resulted in Pco2 levels of 29 ± 6 mmHg, Po2 levels of 94 ± 15 mmHg and, oxygen saturations of 94% ± 4%. Plasma glucose levels were measured with a glucose analyzer (Model 2, Beckman Instruments, Fullerton, CA) and whole blood glucose was measured with Dextrostix® and a reflectance colorimeter (Ames Division, Miles Laboratories). Plasma osmotic pressure was measured with an osmometer (Model OS, Fiske, UXbridge, MA).

The preretinal oxygen tensions were measured using the technique that we have described previously.4 In brief, the pupils were dilated with 10% phenylephrine hydrochloride and 1% cyclopentolate hydrochloride, and a cannula containing the oxygen microelectrode was passed through the pars plana into the vitreous cavity. The cannula and the microelectrode were controlled with micromanipulators. The ocular fundus was observed by axial illumination.
with an operating microscope through a contact lens. We used selected, glass insulated, gold plated, recessed tip microelectrodes (Model 723, Transidyne General Corp., Ann Arbor, MI). These were tapered very slightly with tip diameters of 2 to 4 µm and recess lengths of 5 to 15 µm. The voltage-current relationship (polarogram) of each microelectrode was measured, and a polarization voltage was chosen from the middle of the plateau. The microelectrodes were calibrated in isotonic saline solutions equilibrated at 39.0 °C, with both 100% nitrogen and 5% oxygen in nitrogen, before insertion into the eye and after withdrawal. The selected microelectrode currents were always less than 10⁻¹¹ amp in nitrogen, and the sensitivities were of the order of 7 X 10⁻¹³ amp/mmHg (range, 1.8–12.7 X 10⁻¹³ amp/mmHg).

Although the amplifier (Model 1201, Transidyne General Corp.) and recording system used made it possible to measure the oxygen tension to an accuracy of ±0.1 mmHg, the inevitable electrode drift reduced this accuracy to about ±5 mmHg. The reproducibility of consecutive readings, however, was usually within less than ±1 mmHg. The measurements were made in the vitreous humor facing the area centralis, and the position of the tip of the microelectrode was determined by placing it on the internal limiting membrane of the retina (signaled by a dimpling of the membrane) and then withdrawing it approximately 100 µm.

To test the responsiveness of the retinal circulation to changes in arterial P O₂, the dog's breathing gas was alternated periodically (approximately every 3 min) between room air and 100% oxygen. Throughout this time the preretinal oxygen tension was measured continuously.

After the preretinal oxygen tension values had stabilized, the normoglycemic values were obtained, and then a rapid (30–90 sec) intravenous infusion of glucose was administered as a USP solution of 50% dextrose in water. A dosage of 3 ml/kg was given to each animal. The diabetic animals previously had been given insulin intravenously, as described elsewhere, and glucose was infused in them after their blood levels had dropped into the normal range (<150 mg/dl). The glucose infusion was always given with the animals breathing room air. Both before and after the infusions, the breathing gases were alternated between room air and 100% oxygen. The preretinal oxygen tension transients following each change in breathing gas consisted of an exponential increase or decrease, and then a plateau. Only the plateau values just before and just after the glucose infusion (i.e., within a few minutes of it) are reported herein.

**Results.** The fasting plasma glucose level ± SD of the six diabetic dogs was 491 ± 57 mg/dl, and their plasma osmolality was 313 ± 14 mosmol/kg, compared to the eight normal dogs, whose values were 114 ± 18 mg/dl and 296 ± 8 mosmol/kg. In both groups of dogs starting at normoglycemia, the infusion of glucose resulted in an increase in the plasma glucose levels from normal or below (<150 mg/dl) to about 1500 mg/dl and an increase in plasma osmolality of 10% to 20%. The peaks following the 30 to 90 sec infusion were reached in approximately 2 min and the levels fell with half-times of about 30 min for glucose and 10 min for osmolality. Before the glucose infusion, there was little difference in the plasma levels of glucose and osmolality between the normal and insulin-treated diabetic dogs, and the transients in these following the glucose infusion were also very similar.

Figure 1 is a photograph of a segment of one of the polygraph records from a representative dog (this one happened to be diabetic) obtained before and immediately after its glucose infusion. The intraocular pressure was maintained at 15 mmHg throughout. There was usually a brief, transitory disturbance in the systemic blood pressure. In addition, the pulse pressure increased about twofold while the heart rate remained practically constant. After the blood pressure had returned essentially to baseline, 2 to 3 min, the preretinal oxygen tension was always slightly above the baseline level. The blood pressure had always returned to its baseline value by the time the hyperglycemic steady values of preretinal P O₂ were recorded.

Figure 2 shows the effect of breathing 100% oxygen on the preretinal oxygen tension before and after glucose infusion. Clearly, hyperglycemia increased the effect of breathing 100% oxygen.

The preretinal oxygen tensions, with the dogs breathing room air and 100% oxygen, immediately before and just after the glucose infusion are shown in Table 1. At the times when the preretinal oxygen tensions after the glucose infusion were taken, 2 to 3 min for air breathing and 5 to 6 min for 100% oxygen breathing, they were significantly above the baseline values. For this small group of animals, there was not a statistically significant difference between the data from normal and diabetic animals. Accordingly, the data for all animals were pooled to give the mean values in Table 1. The diabetic animals, however, showed a greater average increase in preretinal oxygen tension when breathing 100% oxygen.

To demonstrate the reproducibility and stability of the preretinal oxygen measurements on both room air and 100% oxygen and, therefore, the significance of the changes shown in Table 1, measurements were made sequentially on one animal (number 5D, diabetic) during the baseline hour preceding its glucose
infusion. The breathing gases were alternated periodically (every 3 or 4 min) from room air (8 values) to 100% oxygen (7 values). The mean preretinal oxygen tensions ± SD for this one animal were 11.6 ± 0.8 mmHg on room air and 49.6 ± 2.9 mmHg on 100% oxygen.

It may be seen from Table 1 that glucose had a significant effect on preretinal oxygen tension in both normal and diabetic animals.

Discussion. The method is limited by the lack of stability of oxygen microelectrodes. Our electrodes were selected, and only about one in five electrodes met our criteria. The polarogram had to have a flat plateau, the nitrogen currents had to be less than $10^{-11}$ amp, the sensitivity less than $1.3 \times 10^{-12}$ amp/mmHg, and the current had to be stable (<10% drift/hr). There was always a tendency for the currents to drift downwards over several hours, and the data were discarded if the calibration, before and after the retinal measurement, differed by more than 25%. This type of oxygen microelectrode can be calibrated in normal saline and reliably used in the vitreous body.4 Vitreous body oxygen tension measurements very close to the retina should reflect the retinal surface oxygen tension both at steady state and during a transient.6

Successful preretinal oxygen tension measurements were made on four normal and four diabetic dogs. The diabetic dogs had been chronically hyperglycemic for over half a year, had twice the normal level of glycosylated hemoglobin (about 10%) and had fasting plasma glucose levels approximately 5 times normal. The diabetic dog is an appropriate if not unique model of the retinal disease, since it has been shown to develop, after a number of years, diabetic retinopathy similar to that seen in man.7 In the present study, however, vascular lesions in the retina were neither expected nor observed. The preretinal oxygen tensions at normoglycemia for the diabetic and normal groups, when breathing room air, were similar to each other and comparable to those found in normoglycemic monkeys and cats.4,6,8 The effects of acute hyperglycemia were similar in the normal and diabetic groups and could be accentuated by periodically giving the animals 100% oxygen to breathe (Table 1).

After the completion of the intravenous infusion of glucose and the return of blood pressure (about 2 to 3 min), the preretinal oxygen tensions were always elevated and the autoregulatory response to breathing 100% oxygen appeared to be impaired. There are two possible explanations for the elevated oxygen tensions: a decrease in utilization of oxygen by the tissue and/or an increase in delivery (blood flow). Hickam and Frayser9 believed that glucose infusion decreased retinal oxygen utilization by the Crabtree effect, increasing tissue available oxygen and hemoglobin saturation. The infusion of glucose might also be expected to cause vasodilatation and an increase in retinal blood flow secondary to the hyperosmolality.10 From our results, it is not possible to determine whether one or both of these two possible explanations is valid.

Rapid changes in preretinal oxygen tensions observed here were similar to oxygen saturation changes observed in normal humans who had been made acutely hyperglycemic2 and to preretinal oxygen tension changes observed in monkeys following the infusion of mannitol.11 Atherton et al reported the ef-
fect of acute hyperglycemia on the retinal circulation of the normal cat. They measured the retinal transit time for fluorescein dye and found that velocity increased following glucose infusion. Their findings also support the hypothesis that hyperglycemia increases blood flow. Surprisingly, the investigators did not show a significant change in retinal arteriole width during glucose infusion. Vessel width was not measured in the studies reported herein, but in comparable human studies, there was significant vasodilation.

In addition to causing an apparent increase in blood flow, acute hyperglycemia also caused a loss of oxygen autoregulation. The inspiration of 100% oxygen is accompanied normally by a marked vasoconstriction and decreased retinal blood flow, termed here oxygen autoregulation. Compared to the normal dogs, the diabetic dogs appeared to have a greater average increase in preretinal oxygen tension both at normoglycemia (49 versus 25 mmHg) and hyperglycemia (75 versus 52 mmHg) (Table 1). It has been reported that diabetic patients have a reduction in their normal retinal vasoconstriction following 100% oxygen inspiration. Possibly this explains the differences observed in Table 1, but the small number of animals studied in each group (four) makes this conclusion less certain. Unfortunately, the unavoidable variability in anesthetic level, and even the type of anesthesia (sodium pentobarbital), tended to partially mask the differences between normal and diabetic dogs.

The present normal and diabetic dog results, cat studies, and observations made in normal and diabetic humans suggest that acute hyperglycemia transiently increases retinal blood flow. It is possible that after the development of diabetic retinopathy, the retinal circulation, while compromised, might none-the-less be maintained by the intermittent hyperglycemic increases in blood flow. We speculate that if patients with diabetic retinopathy were suddenly well controlled, the blood flow might actually decrease, resulting in areas of retinal ischemia and possibly even infarction.

Finally, both hyperglycemia and diabetes appear

Table 1. Preretinal oxygen tension following 100% oxygen breathing, at normoglycemia and hyperglycemia, in normal and diabetic dogs

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Normoglycemia</th>
<th>Hyperglycemia</th>
<th>Effect of O₂ breathing (100% O₂ breathing minus air breathing), mmHg</th>
<th>Effect of hyperglycemia (hyperglycemia minus normoglycemia), mmHg</th>
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<td>36.6*</td>
<td>26.9†</td>
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<td>8.5</td>
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</table>

* Difference is significant P < 0.01.
† Difference is significant P < 0.001.
to impair oxygen autoregulation. It is possible that this, together with the increased blood flow, causes an intermittent stress which might result in permanent vascular damage. This could be one of the earliest pathogenetic events in diabetic retinopathy.

Key words: alloxan, diabetes, diabetic dogs, dogs, glucose, hyperglycemia, hyperosmolality, insulin, oxygen, oxygen tension, retinal circulation, retinal oxygen tension

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References