Retinal Blood Flow During Hyperglycemia

A Laser Doppler Velocimetry Study

Paul M. Sullivan, Geraint E. Davies, Gordon Caldwell, Andrew C. Morris, and Eva M. Kohner

The effect of different rates of glucose infusion on the retinal circulation was studied in Gottingen breed minipigs. Seven minipigs were made hyperglycemic rapidly with an intravenous bolus injection of 50% dextrose, after which a slow dextrose infusion maintained hyperglycemia for 60 minutes. Seven minipigs were more gradually made hyperglycemic over 60 minutes with a slow intravenous infusion of 50% dextrose, and a further seven had a control infusion of urea of equal volume and osmolality over 60 minutes. Retinal blood flow (RBF) was determined from the maximum (centerline) velocity of the blood (V_max) (determined by bidirectional laser doppler velocimetry) and the vessel diameter (D) (determined from monochromatic fundus photographs). Measurements were made in a single temporal retinal vein of each animal at baseline, during, and after each of the infusions. Plasma glucose rose from 6.1 ± 0.5-25.3 ± 1.5 mM (mean ± standard error) during the bolus infusion and from 6.4 ± 0.7-22.0 ± 0.7 mM during the slow infusion. The bolus and the slow glucose infusions both produced large increases in RBF (63% and 62%, respectively) which were mainly attributable to increases in V_max. The urea infusion had no significant effect on RBF, V_max, or D. The ocular perfusion pressure rose slowly and was significantly elevated after 60 minutes of slow glucose infusion but not after the urea infusion.


Several suggest that retinal blood flow (RBF) is increased in diabetes, although not all investigators confirm this. It has been suggested that increased RBF may actually have a detrimental effect on the course of diabetic retinopathy. If RBF is increased in diabetics, it may be a direct effect of hyperglycemia on the retinal circulation.

Previous studies in cats and dogs have found that acute hyperglycemia increases RBF and preretinal PO_2 (the latter being suggestive of increased RBF). In both of these studies extremely rapid changes in blood glucose were induced to levels not normally encountered in human diabetics.

Fallon et al., however, found no change in macular blood flow (measured by blue light entoptoscopy) when human subjects (both diabetic and normal) were rendered hyperglycemic. The degree of hyperglycemia and the rate of glucose elevation were less than those in the animal experiments. Similarly Small et al. did not find any effect of hyperglycemia on RBF in animals. Grunwald et al. showed that normalization of blood glucose by insulin lowered RBF in human diabetics using bidirectional laser doppler velocimetry (BLDV), but since insulin itself is vasoactive, this does not show that hyperglycemia increases RBF.

To explain the apparent discrepancy between these results it was postulated that a slow rise in plasma glucose, as occurs normally in diabetic patients, has less effect on RBF than a very rapid rise. This study was designed to test this hypothesis using the noninvasive and sensitive technique of BLDV.

Materials and Methods

Anesthesia and Preparation of Animals

Twenty-one separate experimental procedures were done involving 14 healthy Gottingen breed minipigs of either sex weighing 5.2-21.3 kg. The animals were treated in accordance with the ARVO Resolution on the Use of Animals in Research. After induction of anesthesia by halothane (ICI, Macclesfield, UK) the animals were intubated, anesthesia being maintained with metamidate (Janssen, Oxford, UK). Once the animals were deeply anesthetized, neuromuscular relaxation was produced with tubocurarine (Duncan Flockhart, Greenford, UK), and the animals were ventilated with a mixture of 30% O_2 and 70% N_2O. The ventilation rate and tidal volume were varied according to the blood gas analysis to
maintain a PO₂ of 110-130 mm Hg and a PCO₂ of 30-40 mm Hg. Cannulas were introduced via the femoral artery into the descending aorta and via the popliteal vein into the inferior vena cava. Arterial samples were regularly taken for blood gas analysis (Ciba-Corning, Halstead, Essex, UK) and plasma glucose analysis using an autoanalyzer (Beckmann, Fullerton, CA). The pulse and blood pressure were recorded continuously using a polygraph (Grass, Quincy, MA). The animals were placed on a thermal mat to maintain a rectal temperature of 37-39°C.

To prepare the minipigs eyes for BLDV 1% cyclopentolate eye drops (Smith and Nephew, Romford, UK) were applied for mydriasis, and lid sutures were inserted to keep the palpebral fissure open and rectus sutures to keep the eye still. A contact lens of appropriate fit was applied using sodium hyaluronate (Pharmacia, Uppsala, Sweden) as a gonio gel to protect the cornea. The pig’s head was supported by a rigid steel frame to prevent transmitted respiratory movement of the head. Intraocular pressure (IOP) measurements were made using a hand-held Perkins applanation tonometer (Clement Clarke International, London, UK). Tonometry was done on the eye not undergoing BLDV because the contact lens prevented applanation. It was assumed that any effect of the hyperosmolar infusions on the IOP would affect both eyes equally. Care was taken to avoid excess traction on the stay sutures because this can also increase the IOP.

Retinal Blood Flow Measurement

Retinal blood velocity was measured using a fundus camera-based bidirectional laser doppler velocimeter (Oculix, Philadelphia, PA). This instrument measures retinal blood velocity by detection of the Doppler frequency shift of low power He–Ne laser light scattered by retinal erythrocytes. Recordings were taken from temporal retinal veins of varying calibres from 140–343 μm, avoiding arteriovenous crossings and vessel junctions. The location of the measurement site was documented by drawing its relationship to the rest of the vascular anatomy. The data were stored on the hard disc of a Masscomp 5000 (Masscomp, Westford, PA) computer for analysis by a masked observer on a separate occasion. Data analysis was fully automated and used an algorithm to determine the high frequency cutoff in the power spectrum from which the maximum (centerline) velocity of the blood (Vmax) was derived. A minimum of 12 spectral pairs, selected on the basis of a form indicating correct alignment of the laser beam on the center of the vessel (ie, with sharp, well-defined spectral cutoffs) were used for analysis.

\[ V_{\text{max}} = k (f_{\text{max}1} - f_{\text{max}2}) \]

where \( V_{\text{max}} \) is the maximum centerline velocity of blood, \( f_{\text{max}1} \) and \( f_{\text{max}2} \) are the maximum frequency shifts in the two channels of the BLDV, and \( k \) is a constant dependent on the wavelength of the laser, the axial length of the minipig eye, the refractive index of the flowing medium, and the geometry of the path of the laser light in the fundus camera.

Vessel diameter at the measurement sites was determined from monochromatic fundus photographs projected onto a graphic digitizing hipad. The mean of 24 readings (ie, six measurements on four different retinal photographs) was taken as the vessel diameter (D).

The RBF was calculated as:

\[ \text{RBF} = \pi D^2/4 \cdot V_{\text{max}}/2 \]

(since \( \pi D^2/4 \) = cross-sectional area of vessel and \( V_{\text{max}}/2 \) = average velocity of erythrocytes).

Experimental Procedure

Hyperglycemia was achieved in seven animals by a 1.75 ml/kg of body weight bolus of dextrose injected over 5 min using a Harvard pump. The infusion rate was then reduced to 0.09 ml/kg of body weight to maintain hyperglycemia for 60 min. Laser Doppler velocimeter readings, fundus photographs, and tonometry were done at baseline and at 5, 15, and 60 min after the start of the infusion. The infusion was then stopped and the plasma glucose allowed to return to baseline values; further readings were taken at 120 min. Where recovery to euglycemia was prolonged beyond 1 hr, intravenous porcine insulin (Ve- losulin, Nordisk, Epsom, UK) 0.15 units/kg of body weight was administered.

In seven animals 50% dextrose was slowly infused to achieve a gradual rise in glucose so that after 60 min the plasma glucose was similar to that attained by the bolus injection. The infusion rate was initially 0.5 ml/min, adjusted every 10 min according to the plasma glucose. Retinal blood flow was measured at baseline and at 30 and 60 min after the start of the infusion and after glucose normalization at 120 min.

In another seven animals a control infusion of urea (BDH, Poole, UK) equiosmolar with the 50% dextrose was administered over the same interval as the slow glucose infusion. Retinal blood flow was measured at the same times as in the slow dextrose infusion, but no readings were taken after normalization of the urea because it took several hours for all the urea to be cleared from the circulation. Blood was taken throughout for subsequent plasma urea determination.
Analysis and Statistics

Perfusion pressure (PP) was calculated as:

$$PP = \frac{1}{2}(BP_{dia} + \frac{1}{2}(BP_{sys} - BP_{dia})) - IOP$$

where $BP_{dia} =$ diastolic blood pressure, $BP_{sys} =$ systolic blood pressure, and $IOP =$ intraocular pressure.

Nonpaired t-tests were used to compare the plasma levels of urea and glucose between the different infusions. Although the RBF data were found to be normally distributed, the increase in variance with increased RBF and a small number of missing data precluded two-way analysis of variance. Linear-regression analysis was therefore used to test the null hypothesis that there was no relationship between the changes in plasma glucose and the changes in RBF seen in the experiments. The regression coefficient of RBF on plasma glucose was determined for each individual experiment using a Minitab statistical software package (Minitab, State College, PA). The 120-min readings were not included in the analysis because the retinal hemodynamic reaction to a fall in plasma glucose might not be a simple reversal of the changes seen during an acute rise. The seven regression coefficients so obtained were tested to see if their mean was significantly different from zero using a one-sample t-test. The changes in $V_{max}$ and D were analyzed similarly.

Because of a difference in baseline RBF in the slow glucose and the rapid glucose infusion, the relationship between baseline RBF and the subsequent change during hyperglycemia was studied. The regression of change in RBF on baseline RBF was determined for both of the glucose infusions using linear-regression analysis.

The PP data were found to be suitable for simple parametric testing (two-way analysis of variance and paired t-tests).

Results

The trends in plasma concentrations of urea and glucose are shown in Figure 1. The bolus injection of glucose produced an immediate and sustained increase in plasma glucose. The slow glucose infusion raised the plasma glucose to similar levels over 1 hr. Although the urea concentrations tended to be slightly higher, especially at the midpoint of the infusion, the final concentrations achieved were not significantly different ($P > 0.05$ by nonpaired t-test).

The retinal vessels measured during the bolus infusion were narrower ($D = 210.1 \pm 12.6 \mu m$ at baseline) than those measured during the slow glucose infusion ($D = 248.6 \pm 15.3 \mu m$). This difference was not significant, but the difference in flow was (RBF = 7.5 \pm 1.3 \mu l/min at baseline in the rapid infusion, RBF = 15.7 \pm 2.9 \mu l/min at baseline in the slow infusion, $P > 0.05$ by nonpaired t-test). The baseline data of the slow glucose and urea infusions were similar.

Hemodynamic Changes After Bolus Hyperglycemia

At 5 min there was a 55% increase in $V_{max}$ ($P < 0.05$) and a small increase in D (3%) which was not statistically significant. The average coefficient of variation for each determination of $V_{max}$ was 30%. The RBF thus increased to 63% above baseline at 5 min ($P < 0.05$) (Fig. 2). The increase in RBF was sustained for the 60-min duration of the infusion. With plasma glucose normalization at 120 min, the RBF approached, but did not reach, baseline values ($n = 6$). There was no correlation between baseline
RBF and the proportional change with hyperglycemia \((r = -0.021, P > 0.05)\). Ocular PP increased slowly to 20% above baseline at 60 min \((\text{BP}_{\text{sys}} \text{ increased 20 mm Hg, BP}_{\text{dia}} \text{ increased 17 mm Hg, and IOP decreased 2.5 mm Hg})\), but this change was not statistically significant (Fig. 3). There was no significant change in the pulse rate.

**Hemodynamic Changes With Slow Infusion of Glucose**

The RBF increased throughout the slow glucose infusion; at 30 min, it was 33\%(n = 6) and at 60 min, 62\% above baseline \((P < 0.01)\) (Fig. 4). As in the bolus injection of glucose, this increase was mainly due to a 48% increase in \(V_{\text{max}}\) \((P < 0.05)\) (Fig. 5). The 5% increase in \(D\) was not statistically significant. With normalization of the plasma glucose the RBF fell back to baseline values \((n = 5)\). As in the bolus injection, the change in flow was not related to baseline RBF \((r = -0.024, P > 0.05)\). Mean ocular PP increased during the infusion (Fig. 3), and at 60 min, it was 14 mm Hg (26%) above baseline \((P < 0.05)\). This increase in PP was partly due to an increase in \(\text{BP}_{\text{sys}}\) (+20.7 mm Hg) and \(\text{BP}_{\text{dia}}\) (+16.3 mm Hg), although there was a small fall in IOP (-1.5 mm Hg). Again, there was no significant change in the pulse rate.

**Hemodynamic Changes With Slow Infusion of Urea**

The urea infusion produced a 20% increase in RBF (Fig. 6), but this was not statistically significant \((P > 0.05)\). Similar small insignificant increases were

**Discussion**

The results of these studies show that hyperglycemia increases RBF. This change occurred after both slow and rapid glucose infusions. Smaller vessels were measured in the bolus experiments and baseline RBF was therefore lower. There was, however, no tendency for vessels with lower baseline RBF to react differently from those with higher baseline RBF during hyperglycemia, so it may be reasonable to compare the proportional change in RBF between the two infusions. On this basis there was no difference between the effects of the two infusions.

The cause of the increase in RBF is not immediately clear. Although an increase in ocular PP was seen it was within the limits for autoregulation in the
normal mammalian retina and occurred after the increase in RBF in the bolus infusion. Keen et al. showed that hyperglycemia increases retinal lactate production in isolated retina, which in vivo could act as a spurious anoxic signal to the retinal circulation leading to retinal hyperperfusion. Alternatively, reduced blood viscosity secondary to hemodilution could increase RBF, although one would expect a similar effect with the urea infusion.

The results presented are similar to those of Atherton et al., who found an increase in RBF due to increased V_max after a bolus glucose infusion in cats using cinefluorangiography. Interestingly neither Atherton’s nor our study showed a significant increase in D during hyperglycemia (the 5% increase in D reported in our study was not statistically significant) as has been reported in humans.

The apparent discrepancy between the results of studies investigating the effect of hyperglycemia on larger retinal vessels and on perifoveal capillaries is not explained by differences in the rate of glucose infusion used. These differences may be attributable to the different responses of the parts of the retina studied to hyperglycemia, the perifoveal retinal circulation maintaining constant local RBF at the expense of the peripheral retinal circulation.

It has previously been shown that insulin-induced reduction of plasma glucose reduces RBF in diabetic patients. In our study an increase in RBF was seen when the plasma glucose was elevated to 14.7 mM, a level of hyperglycemia seen in even reasonably controlled diabetics. Such glucose-dependent fluctuations in RBF in human diabetics may play a role in the pathogenesis of diabetic retinopathy.

Key words: retinal blood flow, laser doppler velocimetry, hyperglycemia, diabetic retinopathy, minipigs

Acknowledgments

The authors thank Margaret Foster and Dennis Wilson for their invaluable technical assistance, Margaret Fitzpatrick for her help with the computer, Neal Alexander for statistical advice, and Steve Aldington and all the members of the Hammersmith grading centre for advice and assistance with the retinal photography.

References