Retinal Blood Flow by Hydrogen Clearance Polarography in the Streptozotocin-Induced Diabetic Rat

Stephen J. Cringle, Dao-Yi Yu, Valerie A. Alder, and Er-Ning Su

Purpose. The authors compared retinal blood flow in rats after 5 weeks of streptozotocin (STZ)-induced diabetes with that in age-matched control animals.

Methods. The flow measurements were based on the hydrogen clearance technique and the intraocular placement microelectrodes at the surface of the retina. The hydrogen was delivered by bolus injection (100 μl) of hydrogen-saturated saline into the ipsilateral carotid artery using a cannula through the lingual artery. The rats were anesthetized and artificially ventilated. Care was taken to match the systemic blood pressure and blood gases in the two groups.

Results. The mean retinal blood flow in the STZ group after 5–6 weeks duration of hyperglycemia was 487 ± 59 ml/min/100 g (standard error) compared with 330 ± 16 ml/min/100 g in the age-matched controls. The variation in retinal blood flow was far more pronounced in the STZ group, even in different locations in the same eye. Changes in fundus appearance were also noted, with second-order arterioles being more apparent and the retina more "pinkish" in appearance in the STZ animals.

Conclusions. The mean retinal blood flow in the region of retina studied in the two groups was significantly higher in the STZ animals than in age-matched controls. The increased heterogeneity of retinal blood flow may reflect a disruption to the normal blood flow control mechanisms in the retina after only 5 weeks of STZ-induced diabetes. Invest Ophthalmol Vis Sci. 1993;34:1716-1721.

Despite the widespread use of the rat model of streptozotocin (STZ)-induced diabetes, there have been few studies of functional changes in the retinal circulation, even though alterations in blood flow and oxygen distribution are likely factors in the progression of the disease. Intracocular microelectrode measurements of oxygen tension in larger animal models of diabetes, such as the dog and cat, found no difference in the mean vitreal oxygen tension compared with that in controls. Although central vitreous oxygen tension was also unchanged in STZ-treated rats, a marked suppression of the normally large oxygen gradients near the retinal arteries was evident. This suggested that a compensatory increase in retinal blood flow may account for the stability of vitreous oxygen tension, despite the apparently lower oxygen tension of the blood entering the eye. We have successfully adapted our intraocular microelectrode technology developed in larger animals to allow rapid and highly reproducible measurements of retinal blood flow using a hydrogen clearance technique in the intact rat eye in vivo.

The principles of the hydrogen clearance technique are well established and have been used to study tissue blood flow in many organs, including the eye. Essentially, the rate of washout of a nonmetabolized and highly diffusible gas from the tissue is di-

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rectly proportional to the blood flow in that region. Despite the fact that the vascular distribution in the eye does not rigidly fit the theoretic requirements, it has been demonstrated that such measurements correlate well with the ocular blood flow measured by direct means in the perfused dog eye. The advantage of the hydrogen clearance method compared with alternatives such as microsphere trapping is that many measurements can be made in the same animal, and the results are available immediately. The flow measurement is also more “local,” with a spatial resolution limited only by the size of the microelectrode tip. The technique therefore has the potential to investigate regional and temporal fluctuations in blood flow in the same animal.

The goal of this study was to determine whether there are any alterations in retinal blood flow in very early diabetes. This was achieved by comparing blood flow in the eye of normal and STZ-induced diabetic rats after only 5–6 weeks of hyperglycemia.

MATERIALS AND METHODS

Animals

Sprague Dawley rats 10–11 weeks of age were randomly assigned into two groups, a control group (n = 10) and an induced diabetic group (n = 8), which received an intraperitoneal injection of STZ 50 mg/kg from a 50-mg/ml solution in sterile water. Initial body weights and blood glucose levels on assignment were 347 ± 12 g (mean and standard error) and 339 ± 9 g and 5.22 ± 0.5 and 6.45 ± 0.35 mmol/l for the control and STZ groups, respectively (Table 1). All rats were housed (two per cage) in plastic containers on sawdust with a 12h:12h light/dark cycle. Ambient light levels averaged 290 lux. They were fed standard laboratory chow with water ad libitum. Diabetes was confirmed in the STZ group after only 5-6 weeks of hyperglycemia.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n)</th>
<th>STZ (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt, (g)</td>
<td>347 ± 12 (10)</td>
<td>339 ± 8.7 (8)</td>
</tr>
<tr>
<td>Wt, (g)</td>
<td>505 ± 20 (10)</td>
<td>344 ± 16 (8)</td>
</tr>
<tr>
<td>BGf (mmol/l)</td>
<td>5.22 ± 0.5 (10)</td>
<td>6.45 ± 0.35 (8)</td>
</tr>
<tr>
<td>BGf (mmol/l)</td>
<td>6.44 ± 1.2 (10)</td>
<td>&gt;22 (8)</td>
</tr>
<tr>
<td>Blood flow (ml/min/100 g)</td>
<td>330 ± 16 (10)</td>
<td>487 ± 59 (8)</td>
</tr>
</tbody>
</table>

Standard errors are used throughout.

Hydrogen Clearance Measurement of Retinal Blood Flow

The equipment and procedures were similar to those described in detail in our initial studies on the validity of the hydrogen clearance technique in the rat eye. The rats were anesthetized with an intraperitoneal injection of 5-ethyl-5-(1'-methyl-propyl)-2-thiobarbiturate (Inactin, Byk Gulden, Konstantz, Germany) 100 mg/kg and atropine sulfate (20 μg). The trachea was cannulated for artificial ventilation. The femoral artery was cannulated for continuous blood pressure monitoring and periodic blood gas sampling, and the lingual artery was cannulated for injection of the hydrogen saturated saline. After this surgery, the rat was placed prone in a modified Stellar stereotaxic instrument (model 51400, Stoelting, ), and the head was fixed in position. The rats were air ventilated at 90 breaths/min with the tidal volume set according to the actual body weight in the case of control rats and the predicted body weight in the case of the STZ rats. This protocol was followed to match the systemic conditions and blood gases closely in the two groups because we had previously established that use of the actual body weight in the STZ group resulted in systemic hypoxia. In the current study, the mean blood gas levels of the STZ group (n = 8) were 88 ± 6 mmHg for oxygen, 36 ± 2 mmHg for carbon dioxide, and a pH of 7.47 ± 0.01, which was directly comparable to that of the large number of control animals studied.

The rectal temperature was also continuously monitored and maintained close to 38°C with an infrared lamp.

The pupil of the operated eye was dilated using 1% tropicamide. The upper eye lid was partially removed and an eye ring was sutured to the conjunctiva at the limbus and fixed to the stereotaxic frame. A diamond knife was used to make a 1.2-mm wide incision through the sclera and uveal tissue in a superior nasal location just less than 2 mm posterior to the limbus. The microelectrode was then placed into the vitreous body through this entry hole. All procedures conformed to the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research.

Glass-insulated platinum-rhodium microelectrodes were manufactured in our own laboratory.
with a typical tip diameter of 5 μm and approximately 5 μm of exposed length. No membrane was applied. Hydrogen sensitivity was tested in a calibration bath before use. After insertion into the eye, the electrodes were biased at +0.3 V with respect to a reference electrode of silver–silver chloride (placed subcutaneously at the central vertex), and the hydrogen-generated current was measured using a picoammeter (480 Pico, Keithley, ). The combination of direct visual examination of the fundus (operating microscope and plano-concave contact lens), the angular adjustment of the stereotaxic frame, and the piezoelectric translation of the microelectrode (1-μm steps) allowed highly reproducible placement of the electrode tip on the retinal surface at the chosen location (usually in the inferior retina two or three disc diameters from the optic disc margin).

We developed our own computer-controlled microinjection system,6 capable of highly reproducible injections of a selected volume of hydrogen-saturated saline at a given rate and with a timing accuracy of better than 0.1 sec. The optimum injection volume was found to be 100 μl at 100 μl/sec, which produced easily recordable responses without noticeable disruption to the stability of the animal or its fluid balance during the course of the experiment.

The monitored systemic parameters and the hydrogen clearance current were accessed by the computer using an analog to digital conversion (RT1815, Analog Devices). A schematic of the experimental arrangement is shown in Figure 1. The hydrogen current was sampled for 40 sec at 0.1-sec intervals, which represented a good compromise between the temporal resolution required and the quantity of data generated. After each clearance measurement, the data were analyzed and displayed graphically on line, together with a semilog plot of the clearance phase and calculated values for the flow rate and linear-regression coefficient. The raw data were also stored for further analysis and hard-copy plotting.

**Experimental Protocol**

After the animal’s condition was stable and the microelectrode was positioned in the eye, an initial injection of hydrogen-saturated saline was given to establish cannula patency and check visually the arrival of the bolus at the eye. The electrode tip was then placed at the chosen retinal location, and hydrogen clearance curves were recorded for subsequent injections. All experiments were performed in photopic conditions. At the conclusion of the experiment, the rat was killed with an anesthetic overdose.

![Figure 1](https://iovs.arvojournals.org/photoinclusions/image hướng dẫn/933398/04/30/2018)
RESULTS

Ocular and Systemic Changes Observed at the Time of the Acute Experiment

At 5–6 weeks after the STZ induction, most of the STZ rats had a mild cortical cataract, which did not interfere significantly with the optical view of the fundus. The normally transparent retina was noticeably pinkish in the STZ rats, and the retinal arterioles were clearly dilated. It was noticed during preparatory surgery that the connective tissue of the STZ rats was sticky and friable and also that the jugular vein was dilated compared with that of normal rats.

Systemic Conditions

Table 1 summarizes the means and standard errors of some of the physiologic parameters for the control and the STZ groups, including the initial body weight and blood glucose values at induction and the equivalent final values at the time of the acute experiment. It is apparent that there was no significant difference in body weight and blood glucose at induction. However, as already stated, by the time of the acute experiment, the body weight was significantly less in the STZ group, and the blood glucose was elevated to greater than 22 mmol/l, which is the upper limit of the Ames glucometer.

Retinal Blood Flow

Figure 2 shows a typical hydrogen clearance curve together with the semilog plot of the clearance phase for an STZ rat. The flow rate is calculated from the slope of the semilog plot of the clearance phase. Only data points either side of the half-amplitude point and covering a total time span equal to the half-time are used. This effectively excludes the early and late stages of the clearance where nonlinearity or poor noise immunity may be a problem. In the example shown, the region used in the calculation is shown between the dashed lines. Figure 3 summarizes the calculated flow data for the control and STZ groups. The grand means and standard errors of retinal blood measurements were 330 ± 16 ml/min/100 g (standard error, n = 10) and 487 ± 59 ml/min/100 g (n = 8) for the control and STZ group, respectively. Because the standard errors of the two groups were significantly different (variance ratio test), a modified t test was used to test for the significance of difference of the means of these two groups. The blood flow in the STZ group was significantly higher than in the control group (P < 0.025).

DISCUSSION

These data demonstrated a 47% increase in mean retinal blood flow and a greater heterogeneity 5–6 weeks after the onset of STZ-induced diabetes. Inspection and comparison of fundus appearance in the two groups showed a more pinkish fundus in the STZ rats, with second-order arterioles and venules being much more easily visualized. This might imply that these ves-
The larger systemic veins were clearly dilated in the diabetic rats, which may imply a larger blood volume. It should be noted that retinal blood flow was often markedly different in adjacent areas of retina in the STZ animals, which is reflected in the large standard error of the measurement in this group. This may be related to the visible fundus changes and the likely disruption of the normal regional control mechanisms resulting in a redistribution of retinal blood flow. Such marked regional variations were not seen in the control animals. In the current study, we were not able to determine whether the observed changes were a direct consequence of the induced diabetes or a result of the chronic hyperglycemia. Future experiments are proposed in which the effect of insulin control will be determined.

An increase in blood flow has recently been reported in the retina of STZ rats of 6 weeks' duration of diabetes (measured by the microsphere method).1 In that study, the blood flow was increased by 20% in the diabetic retina, but the reported flow rates of 43 ml/min/100 g tissue for normal rats was far below the values measured in the current study. The quantitative accuracy of the various techniques for measuring retinal blood flow is hard to assess. We have previously demonstrated the linearity of our hydrogen clearance technique for choroidal blood flow measurements in the perfused dog eye, in which the total ocular flow is known and can be manipulated at will to cover the entire physiologic range.10 We have also made measurements of retinal blood flow in the dog and, more recently, have made intraretinal measurements in the rat. It was demonstrated that the contributions from both the retinal and choroidal circulations can be distinguished.6

By contrast with the findings in the rat, microsphere studies in dogs demonstrated a decrease in retinal blood flow of more than 40% from a control value of 91 ml/min/100 g after 5 mo of alloxan-induced diabetes.13 This dichotomy of results may reflect the difficulty of comparing measurements from different animal models and at different stages of the disease. There is more agreement about the the effect of diabetes on the autoregulatory capacity of the retinal circulation. In human diabetic patients, the autoregulatory response to hyperoxia was suppressed,14 and in oxygen studies in diabetic dogs, the inner retinal oxygen tension showed a much larger rise with systemic hyperoxia.9 Thus, other independent measurements suggest disruption of retinal blood flow control in early diabetes. The heterogeneity of retinal blood flow evident in the STZ rat is particularly interesting, and we intend to employ the hydrogen clearance technique to study in more detail the spatial and temporal distribution of retinal blood flow to obtain a better understanding of the control mechanisms involved.

There was no clear evidence of pathologic condition in the whole mounts of the retinal vasculature prepared by trypsin digestion after 5–6 weeks of STZ-induced diabetes. However, significant changes were observed by 20–30 weeks, which were consistent with many other histologic studies of diabetes,15 including endothelial cell proliferation in some areas, loss of endothelial cells and pericytes in other areas, basement membrane thickening, and dilatation of retinal vessels mainly on the venous side. Such changes are thought to be the direct result of the hyperglycemia, which is a basic feature of diabetes, and causes a disturbance of the polyol pathway, mainly in the mural cells of the vasculature. Similar histologic changes have also been shown to occur in the galactosemic rat model,16 in which the polyol pathway is also overloaded.

The well-established rat model of diabetes, the STZ-induced diabetic rat, has been reported to develop pathologic changes in the microvasculature comparable to those seen in human diabetic patients.15,16 The ability to make measurements of retinal blood flow in such a convenient model of diabetes may be important in determining the relationship between various stages of the pathologic findings with functional properties of the retinal circulation. This would be difficult to achieve in larger animal models of diabetes or in humans. The increased technical requirements for microelectrode placement in the 55-μl vitreal cavity of the rat eye is more than offset by the convenience and growing importance of rat models of vascular disease.

Key Words
retina, blood flow, diabetes, streptozotocin, microelectrodes

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