Effects of Insulin-Induced Acute Hypoglycemia and Normoglycemic Hyperinsulinemia on the Retinal Uptake and Ocular Metabolism of Glucose in Rabbits

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Glucose is the principal metabolic substrate for the retina in mammals, being essential for maintaining the functional activity of the retina; it can be supplied to the tissue by both vitreous humor and blood. Yet, the impact of hypoglycemia on retinal glucose metabolism has been poorly investigated. We have therefore studied the effects of acute insulin-induced hypoglycemia on the glucose uptake and metabolism in the retina, by analyzing the hypoglycemia-induced changes in the ocular distribution and metabolic fate of [^{3}H]-2-deoxy-D-glucose (2-DG) and [^{14}C]-D-glucose, both injected in the vitreous body. Rabbits were rendered hypoglycemic by subcutaneous injection of insulin (0.8 and 1.2 IU/kg). Insulin-induced hypoglycemia increased both retinal [^{3}H]-radioactivity levels and retina to vitreous humor ratio of [^{3}H]-radioactivity levels ([^{3}H]-[R/VH]). Radio-chromatography showed that hypoglycemia did not induce any change in the retinal conversion of 2-DG to 2-DG-6-phosphate, but increased the conversion of [^{14}C]-D-glucose to [^{14}C]-lactate. Normoglycemic hyperinsulinemia caused no change in either retinal [^{3}H]-radioactivity levels or [^{3}H]-[R/VH] while decreasing retinal [^{14}C]-radioactivity levels and retina to vitreous ratios of ^{14}C-radioactivity levels. These results indicate that acute hypoglycemia increases the uptake rate of glucose by the retina and suggest that normoglycemic hyperinsulinemia may decrease retinal lactate, possibly stimulating its removal from the retina.

GLUCOSE IS THE principal metabolic substrate for the retina in mammals,[1-3] being essential for maintaining the retinal electrical activity in vitro.[3, 4] Yet, the impact of hypoglycemia on retinal glucose metabolism has been poorly investigated. Some findings suggest that hypoglycemia may induce acute adaptative changes in retinal metabolism. Thus, in humans, acute mild hypoglycemia caused a luminance-electroretinogram b-wave amplitude increase similar to that induced by an augmentation of glycemia.[5] In the perfused cat eye, low glucose decreased the electroretinogram-b-wave amplitude,[6] an effect that has been interpreted as a sign of increased glucose utilization by the Muller cells.[7] In rabbits, insulin-induced hypoglycemia appeared to increase the consumption rate of vitreous humor glucose[8]; because vitreal glucose is mainly consumed by the retina,[9,10] this finding suggested that hypoglycemia may increase the retinal uptake of glucose. This hypothesis has not been confirmed and the effects of insulin-induced hypoglycemia on retinal glucose metabolism have not been further characterized. In this study, we have therefore investigated the effects of insulin-induced hypoglycemia on the uptake and metabolism of glucose in the retina and other ocular tissues of rabbits. In their in vivo study, Berkowitz et al[10] have shown that the rabbit retina takes up intravitreally injected 2-deoxy-D-glucose (2-DG) and metabolizes intravitreally injected D-glucose; and that the uptake and metabolism by the retina is the dominant route of the clearance of vitreal 2-DG and D-glucose. On the basis of these findings, we studied the influence of insulin-induced hypoglycemia on the glucose uptake and metabolism in the retina, and other ocular tissues, by analyzing how hypoglycemia affects the ocular distribution and metabolic fate of labelled 2-DG and D-glucose, both injected in the vitreous body.

Insulin has been shown to stimulate the uptake of 2-DG from the whole rat retina in vitro[11] and to enhance the electrophysiologic effect of low glucose in the perfused cat eye.[6] Insulin receptors have been found in the neural retina,[12,13] in retinal blood vessels,[14] and in retinal pigment epithelium.[15] They are also present in the lens,[16,17] iris, ciliary body, and cornea.[18] An increase of plasma insulin might therefore affect ocular metabolism of glucose, considering that insulin can cross the blood-retina barrier.[19] We have therefore verified whether an increase of the plasma insulin levels similar to that observed in hypoglycemic rabbits affected per se the ocular distribution and metabolic fate of labelled 2-DG and D-glucose by using an euglycemic hyperinsulinemic clamp technique.

MATERIALS AND METHODS

Animals and General Procedures

Adult, male, albino New Zealand rabbits weighing 3.0 to 3.3 kg each were used. All rabbits were fasted 16 hours before the experiments. The rabbits were anesthetized with intraperitoneal pentobarbital. Both the central ear arteries were cannulated using a heparinized polyethylene tubing. The catheters were flushed with a heparin solution. Blood pressure was recorded from 1 arterial cannula with a pressure transducer, calibrated by means of a standard sphygmomanometer. Blood samples were withdrawn from the other arterial cannula. Plasma glucose was measured using a glucose analyzer II (Beckmann Instruments, Fullerton, CA). PaO_2, $PaCO_2$, and pH were measured on a microelectrode system (Corning Medical, Medford, MA). Body temperature was maintained at 38°C to 39°C throughout the experiments. Twenty mi-

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croliters of saline containing 4.625 kBq or 9.250 kBq ^{3}H-labeled-2-DG ([^{3}H(G)]2-deoxy-D-glucose, specific activity: 259 GBq/mmol, DuPont NEN, Boston, MA) and 37.0 kBq ^{14}C-labeled-D-glucose ([^{14}C(U)]-D-glucose, specific activity 9.805 GBq/mmol, DuPont NEN) were injected intravitreally. To determine baseline values, 2 blood samples were withdrawn before and after intravitreal injection at a 15-minute interval. Additional blood samples were generally withdrawn each 10 minutes both from treated and control rabbits. Before enucleating the eyes, an additional 25 mg/kg pentobarbital was injected intravenously. After enucleation, the aqueous humor was withdrawn with a syringe. The eyes were then placed in cup-shaped holders. The front portion of the eye was sliced off just posterior to the ora serrata and removed together with the vitreous humor, which adhered to the lens and ciliary body. The posterior emisphere was then turned inside out, and the retina was gently teased free with a smooth glass spatula. The choroid was then detached from the sclera. Retina and choroid were transferred in preweighed tubes and immediately frozen in liquid nitrogen. Retinas were frozen within 30 seconds and choroids within 45 seconds after enucleation. In the meantime, the anterior segment tissues, ie, cornea (the sclera adjacent to the cornea was cut and added to the anterior sclera portion), iris, ciliary body, and lens were collected. The sclera was cleaned from adhering tissues and cut in 3 portions (anterior, mid, and posterior). These tissues were blotted, weighed, and solubilized with Soluene-350 (Packard, Perkin Elmer, Boston, MA). Vitreous humor was collected and homogenized at 0°C. Solubilized tissues were added with 10 mL of a liquid scintillation cocktail (Packard Ultima Gold, Perkin Elmer) and the radioactivity was measured with a liquid scintillator β-counter (Packard Tri-Carb 2100TR, Perkin Elmer). Radioactivity of plasma (500 μL), aqueous humor (100 μL), and vitreous humor (250 μL) was determined directly in Ultima Gold (10 mL).

The rabbits were kept, treated, and killed in accordance with principles of laboratory animal care and the Italian Ethics governing these experiments.

Untreated Control Groups

In preliminary experiments, 6 groups of 2 to 3 rabbits received intravitreally 9.250 kBq ^{3}H-labeled-2-DG ([^{3}H]-2-DG) and 37.0 kBq ^{14}C-labelled-D-glucose (^{14}C-D-glucose); the eyes were enucleated 24, 45, 75, 120, 150, or 270 minutes after intravitreal injection. On the basis of the results of these preliminary experiments, we selected the 75-minute exposure interval for studying the effects of hypoglycemia on the distribution and metabolism of the labeled compounds. Therefore, additional 75-minute control values were subsequently obtained from 2 rabbits injected with 9.250 kBq [^{3}H]-2-DG (these 2 rabbits also received intravitreally 37.0 kBq ^{14}C-D-glucose) and 3 rabbits injected with 4.625 kBq [^{3}H]-2-DG (these 3 rabbits also received intravitreally 18.5 kBq ^{14}C-D-glucose). In summary, 75-minute controls were as follows: 5 rabbits intravitreally injected with 9.250 kBq [^{3}H]-2-DG and 37.0 kBq ^{14}C-D-glucose; 3 rabbits intravitreally injected with 4.625 kBq kBq [^{3}H]-2-DG and 18.5 kBq ^{14}C-D-glucose. All these 8 rabbits served as controls for: vitreous glucose, lactate, and insulin concentrations; plasma glucose and insulin; mean arterial blood pressure, PaO_2, $PaCO_2$, and arterial pH.

Insulin-Induced Hypoglycemia

To investigate the effects of different degrees of hypoglycemia, we used 2 insulin doses, 1.2 and 0.8 IU/kg. In preliminary experiments, we found that after the 1.2 IU/kg dose, glycemia gradually decreased to about 2 mmol/L and hypoglycemia lasted more than 2 hours. Under these conditions, [^{3}H]-2-DG and [^{14}C]-D-glucose could be injected intravitreally when hypoglycemia was already well established (ie, 60 minutes after insulin administration) because the hypoglycemic period was long enough to allow elapsing the 75-minute exposure to the radioactive tracers before increasing of glycemia. The 0.8 IU/kg dose caused a milder hypoglycemia, which also lasted less, because glycemia began increasing 60 to 80 minutes after insulin administration; hence, with the lower dose, [^{3}H]-2-DG and [^{14}C]-D-glucose had to be injected intravitreally before insulin administration, to allow elapsing the 75-minute exposure to the radioactive tracers before increasing of glycemia. Two distinct protocols (A and B) were therefore adopted.

Protocol A. Crystalline porcine insulin (potency 27 IU/mg, Sigma Chemical, St Louis, MO) was administered subcutaneously (1.2 IU/kg) to 7 rabbits. Blood samples were withdrawn from the central ear artery each 10 minutes after insulin administration. [^{3}H]-2-DG (4.625 kBq, 3 rabbits, or 9.250 kBq, 4 rabbits) and [^{14}C]-D-glucose (18.5 kBq, 3 rabbits, or 37.0 kBq, 4 rabbits) were injected intravitreally 60 minutes after insulin administration. The eyes were enucleated 75 minutes after intravitreal injection and ocular tissues collected and treated as described above.

Protocol B. In a group of 10 rabbits, [^{3}H]-2-DG (9.250 kBq) and [^{14}C]-D-glucose (37.0 kBq) were injected intravitreally 15 minutes before administering subcutaneously 0.8 IU/kg of insulin. Blood samples were withdrawn from the central ear artery each 10 minutes after insulin administration and plasma glucose measured. The eyes were enucleated 75 minutes after intravitreal injection. In the preliminary experiments, we had also observed that the hypoglycemic response of rabbits receiving 0.8 IU/kg of insulin was markedly more variable than that of rabbits receiving 1.2 IU/kg. Because the retinal response could possibly relate to the degree of hypoglycemia, we classified as "high responders" those rabbits whose glycemia values were below 2.8 mmol/L for at least 2 consecutive determinations, ie, for at least 10 minutes, and as "mild responders" the other rabbits (2.8 mmol/L is the threshold glycemic value for the symptoms of hypoglycemia and plasma norepinephrine increment in humans[20] and plasma epinephrine increase in rats.[21] According to these criteria, 6 rabbits were classified as "high responders", and 4 as "mild responders".

Hypoglycemia With Maintenance of Vitreous Glucose Levels

Because in hypoglycemic rabbits vitreous glucose decreased with respect to control values (see Results), in these rabbits the vitreal [^{3}H]-2-DG to endogenous glucose ratio became gradually higher than the control value. Theoretically, this might contribute to increase, in hypoglycemic rabbit eyes, the tissue [^{3}H]-radioactivity levels and tissue to vitreous ratio of [^{3}H]-radioactivity levels (D-glucose might act as a competitive inhibitor of 2-DG uptake, see Kletzien and Perdue[22]). To check this possibility, we performed experiments in which the decrease of vitreous glucose was prevented by injecting intravitreously D-glucose. Four rabbits were injected in 1 eye with [^{3}H]-2-DG (9.25 kBq) and [^{14}C]-D-glucose (37 kBq) dissolved in 20 μL of a 5% D-glucose solution, thus also receiving 1 mg of intravitreal D-glucose; the contralateral eye received [^{3}H]-2-DG and [^{14}C]-D-glucose (9.25 kBq and 37 kBq, respectively) dissolved in 20 μL saline. Subcutaneous insulin (1.2 IU/kg) was administered 15 minutes after intravitreal injection. The eyes were enucleated 75 minutes after intravitreal injection and ocular tissues collected and treated as described above.

Normoglycemic Hyperinsulinemia

The marginal ear vein of 3 rabbits was cannulated and connected to an infusion pump. Two blood samples were then collected to determine baseline glycemia. Insulin (1.2 IU/kg) was administered subcutaneously. Ten minutes after insulin administration, infusion of a 20% glucose solution started at a rate of 0.6 mL/kg/h. Infusion rate was then progressively increased to 1.7 to 2.7 mL/kg/h to maintain glycemia, as previously described[23]; blood samples were withdrawn and plasma glucose measured each 5 minutes. [^{3}H]-2-DG (9.250 kBq) and [^{14}C]-D-glucose (37.0 kBq) were injected intravitreally to both eyes 60 minutes after insulin administration. The eyes were enucleated 75 minutes after the intravitreal injection.

Radiochromatography of Vitreous, Retina, and Choroid Extracts

Aliquots of vitreous humors (250 μL) were added with $HClO_4$ 6 N (83 μL) and centrifuged at 20,000 *g* for 10 minutes. Surnatants were separated by high-performance liquid chromatography (HPLC), using an Aminex HPX-87H (300 × 7.8 mm) anion exchange column (Bio Rad, Hercules, CA) with H_2SO_4 5 mmol/L as the mobile phase, flow = 0.3 mL/min, temperature 55°C. One-minute fractions were collected; the fractions were then added with 10 mL Ultima Gold and their radioactivity measured. Retinas and choroids were added with 800 and 350 μL water (HPLC grade), respectively; and the tissues were homogenized by sonication at 0°C with a Model 60 sonic dismembrator (Fisher Scientific, Pittsburgh, PA). Aliquots of the resulting suspensions were added with $HClO_4$ 6 N and then treated and analyzed like the vitreous samples.

Analysis of Plasma Insulin

Immunoreactive insulin concentrations were determined with a commercially available coated tube radioimmunoassay kit (Technogenetics, Milan, Italy).

Analysis of Vitreous Glucose, Lactate, and Insulin

Glucose concentrations of fresh vitreous samples were measured with the hexokinase/glucose-6-phosphate dehydrogenase method; calibration curves were obtained using freshly prepared glucose solutions in saline. Lactate concentration was measured with a commercial lactate dehydrogenase kit (Sigma Chemical) that couples lactate to the reduction of nicotinamide adenine dinucleotide (NAD). Vitreous insulin was determined as previously described[19]; vitreous humor samples were transferred to ultracentrifuge tubes containing a solution of protease inhibitors (leupeptin 0.27 mmol/L, chymostatin 0.02 mmol/L, pepstatin 0.14 mmol/L, and bestatin 0.05 mmol/L, all from Sigma) and centrifuged at 30,000 *g* for 20 minutes; the surnatants were collected and stored at −80°C until used. Immunoreactive insulin concentrations were determined with a commercially available coated tube radioimmunoassay kit (Technogenetics).

Statistical Aalysis

Ocular data (vitreous glucose and lactate concentrations, tissue radioactivity levels, tissue to vitreous ratios of radioactivity levels, percentages of [^{3}H]-2-DG-6-phosphate and [^{14}C]-lactate) of each rabbit were calculated by averaging the values found in the 2eyes. Therefore, n = number of rabbits, with the exception of the "hpoglycemia with maintenance of vitreous glucose levels" experiment, where n = number of eyes, because the 2 eyes were treated differently. Data are presented as means ± SD. Statistical significance of the differences between groups was evaluated by Student's *t* test or 1-way analysis of variance (ANOVA), followed by Student-Newman-Keuls test, as appropriate.

RESULTS

Distribution of Radioactivity and Metabolic Fate of Labeled Compounds in the Eyes of Control Rabbits

[^{3}H]-radioactivity. In preliminary experiments, time-courses (25 to 270 minutes after the intravitreal injection of 9.250 kBq [^{3}H]-2-DG) of [^{3}H]-radioactivity levels in the ocular tissues of control rabbits were determined. Time-courses of [^{3}H]-radioactivity levels in vitreous humor and retina are shown in Fig 1A. Retinal levels were higher than vitreous ones from 25 minutes onward. The values of retina to vitreous ratio of radioactivity levels ([^{3}H]-[R/VH]) were constant between 45 and 120 minutes after the intravitreal injection and increased thereafter (Table 1). Therefore, the 75-minute exposure interval was chosen to study the effect of the various experimental conditions (hypoglycemia, normoglycemic hyperinsulinemia, etc) on the radioactivity distribution to minimize the influence of time on [^{3}H]-[R/VH]. In all other ocular tissues, [^{3}H]-radioactivity levels were always lower than vitreous ones; they also peaked at 45 minutes, like in the retina, but the subsequent decrease was faster than that observed in the retina. In the lens, however, radioactivity levels were approximately constant from 25 to 270 minutes (data not shown).

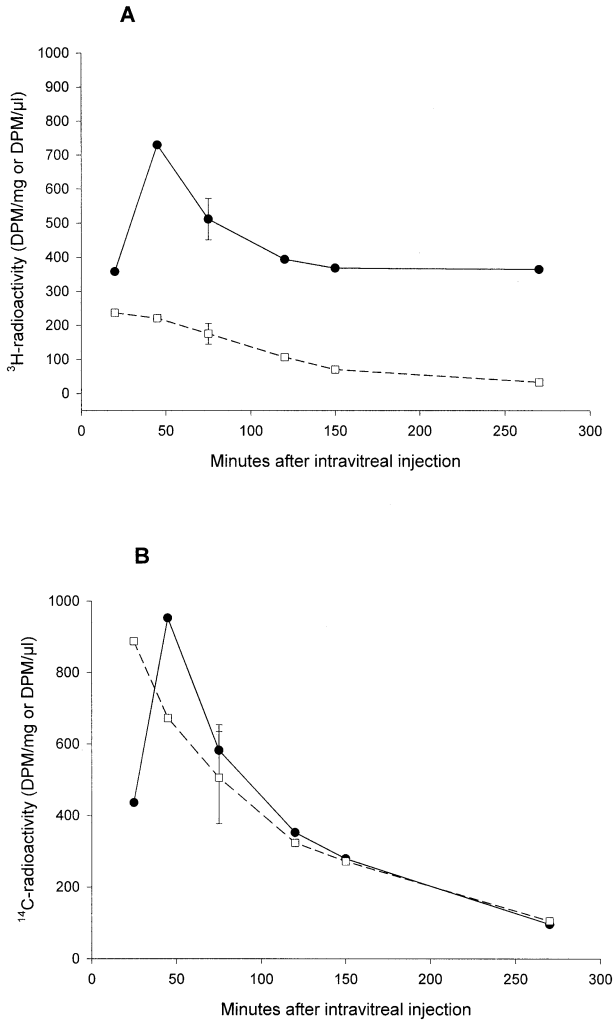

Fig 1. Time-courses of (A) ^{3}H and (B) ^{14}C radioactivity levels in the vitreous humor (□) and retina (●) of control rabbits after the intravitreal injection of [^{3}H]-2-deoxyglucose (9.25 kBq) and [^{14}C]-D-glucose (37 kBq).

Radiochromatography showed that the radioactivity found in the vitreous humor 75 minutes after the intravitreal injection was entirely due to [^{3}H]-2-DG. At the same time, [^{3}H]-2-DG-6-phosphate represented 47% ± 9% (mean ± SD, n = 5) of total radioactivity in the retina and 28% ± 8% (mean ± SD, n = 5) in the choroid, the remaining being [^{3}H]-2-DG.

[^{14}C]-radioactivity. Figure 1B shows the time-courses of radioactivity levels in vitreous humor and retina after intravit-

Table 1. Values of the Retina to Vitreous Ratio of [^{3}H]-Radioactivity Levels ([^{3}H]-[R/VH]) at Different Times After Intravitreal Injection of 9.25 kBq [^{3}H]-2-DG

45 Min	75 Minutes	120 Minutes	150 Minutes	270 Minutes
3.4 (2)	3.1 ± 0.3 (5)	3.9 (2)	5.4 (2)	11.8 (2)

NOTE. Values are means (±SD for the 75 minutes value). Number of samples is shown in parentheses.

real injection of 37.0 kBq of [^{14}C]-D-glucose. After an initial peak at 45 minutes, retinal levels tended to coincide with the vitreous ones. Radiochromatography showed that the radioactivity found in the retina 75 and 120 minutes after the intravitreal injection was entirely due to [^{14}C]-lactate, while in the vitreous humor [^{14}C]-lactate accounted for 19% ± 2% (mean ± SD, n = 5), 75 minutes after intravitreal injection, and 24% (n = 2), 120 minutes after the injection, of the total radioactivity, the remaining being [^{14}C]-D-glucose. Choroid and anterior sclera radioactivity levels also peaked at 45 minutes and decreased thereafter, but were always lower than vitreous and retinal levels (not shown); in the choroid, [^{14}C]-lactate accounted for 73% ± 5% (mean ± SD, n = 5) of total radioactivity, the remaining being [^{14}C]-D-glucose, 75 minutes after the intravitreal injection. In the iris and ciliary body, as well as in mid and posterior sclera and cornea, the time-courses were different, decreasing with an approximately constant rate from 25 to 270 minutes. In the lens, radioactivity levels were approximately constant from 25 to 270 minutes (data not shown).

In control rabbits, glycemia never differed significantly from basal value (5.93 ± 0.65, mean ± SD, n = 8); plasma insulin never increased significantly above basal value (11 ± 6 μIU/mL, mean ± SD, n = 8).

Insulin-Induced Hypoglycemia

[^{3}H]-Radioactivity

Protocol A. The subcutaneous injection of 1.2 IU/kg insulin caused a gradual decrease of glycemia; 60 minutes after injection, when labeled 2-DG (4.625 or 9.250 kBq) and [^{14}C]-D-glucose (37.0 or 18.5 kBq) were injected intravitreally, glycemia was about 2.35 mmol/L (ie, about 40% of basal value) and remained between 2.2 and 1.8 mmol/L up to 75 minutes after intravitreal injection (ie, 135 minutes after insulin), when the eyes were enucleated (Fig 2). Vitreous glucose concentration in the treated group was 1.55 ± 0.22 mmol/L (mean ± SD, n = 7), significantly ($P < .001$) lower than the concentration of the control group, which was 2.89 ± 0.28 mmol/L (mean ± SD, n = 8). Vitreous lactate concentration in the treated group was 13.4 ± 1.1 mmol/L (mean ± SD, n = 7), not significantly different from the concentration in the control group, which was 12.8 ± 0.7 mmol/L (mean ± SD, n = 8). After insulin administration, mean plasma insulin levels increased to above 200 μIU/mL (Fig 2). Hypoglycemia caused no significant change in mean arterial blood pressure (MABP), PaO_2, $PaCO_2$, and arterial pH (data not shown).

In the eyes injected with 9.250 kBq [^{3}H]-2-DG, [^{3}H]-radioactivity levels of insulin-treated rabbits were significantly higher than control levels in the retina (+ 73%) (Table 2).

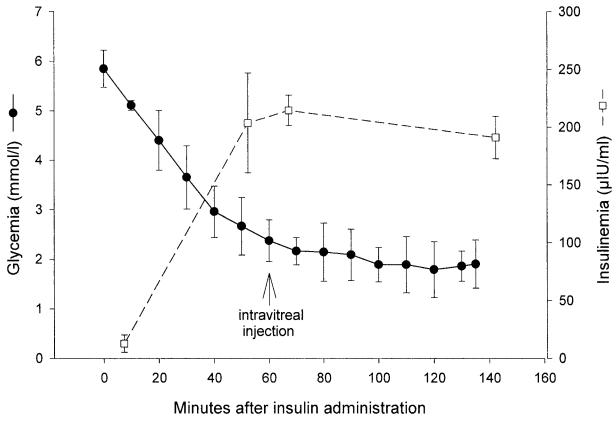

Fig 2. Time-courses of glycemia (●) and insulinemia (□) in a group of 7 rabbits treated with subcutaneous insulin (1.2 IU/kg; Protocol A). Data are means ± SD.

[^{3}H]-[R/VH] of insulin-treated rabbits was 6.7 ± 1.2 (mean ± SD, n = 4), significantly ($P < .001$) higher than the control value (3.1 ± 0.3, mean ± SD, n = 5). In the other ocular tissues, neither radioactivity levels (Table 2) nor tissue to vitreous ratios (not shown) were significantly altered by insulin-induced hypoglycemia. Radiochromatography showed that in hypoglycemic rabbits the percentages of total radioactivity due to of 2-DG-6-phosphate in the retina (52% ± 11%, n = 4, mean ± SD), choroid (23% ± 6% n = 4, mean ± SD), and vitreous (absent) did not differ significantly from those of control rabbits.

In the eyes injected with 4.625 kBq [^{3}H]-2-DG, [^{3}H]-radioactivity levels of insulin-treated rabbits were higher than controls in the retina (controls: 171.1 ± 12.4 dpm/mg; insulin-treated: 325.7 ± 6.83 dpm/mg; means ± SD, n = 3 for both groups, $P < .001$), but not in the other ocular tissues (not shown). Insulin-induced hypoglycemia also caused a significant increase of [^{3}H]-[R/VH] (Fig 3). It should be noted that [^{3}H]-[R/VH] values were not significantly influenced by the

Table 2. [^{3}H]-Radioactivity Levels (dpm/mg or dpm/μL) in Ocular Tissues in Control and Insulin-Treated (Protocol A) Rabbits, 75 Minutes After the Intravitreal Injection of 9.25 kBq [^{3}H]-2-DG

Tissue	Controls (5)	Insulin-Treated Protocol A (4)
Vitreous humor	175.5 ± 30.7	137.5 ± 18.7
Retina	511.8 ± 60.7	884.9 ± 86.7*
Choroid	92.1 ± 15.9	136.3 ± 52.7
Ciliary body	61.1 ± 13.9	63.6 ± 16.5
Iris	58.2 ± 16.9	56.8 ± 14.4
Lens	58.6 ± 35.4	48.4 ± 9.8
Anterior sclera	71.3 ± 8.7	77.5 ± 20.2
Mid sclera	27.7 ± 8.2	23.8 ± 4.01
Posterior sclera	25.0 ± 2.2	20.0 ± 4.3
Cornea	16.7 ± 6.1	10.1 ± 1.4
Aqueous humor	3.3 ± 0.8	2.5 ± 0.90

NOTE. Values are means ± SD. Number of rabbits is shown in parentheses.

*$P < .001$ *v* controls.

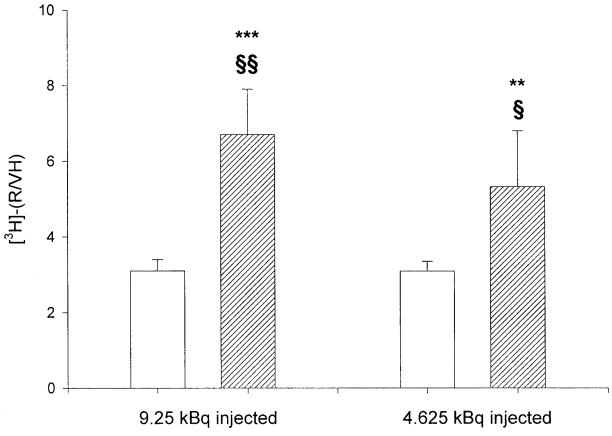

Fig 3. Retina to vitreous ratios of [^{3}H]-radioactivity levels in control (□) and Protocol A insulin-treated (▨) rabbits, 75 minutes after the intravitreal injection of 9.25 kBq or 4.625 kBq [^{3}H]-2-deoxyglucose. Data are means ± SD (experiments with 9.25 kBq injected: n = 5 for control, n = 4 for treated groups; experiments with 4.625 kBq injected: n = 3 for both control and treated groups). **P < .01 and ***P < .001 *v* 9.25 kBq controls. §P < .05 and §§P < .01 *v* 4.625 kBq controls.

amount of [^{3}H]-2-DG injected, both in control and hypoglycemic rabbits.

Protocol B. The time-course of glycemia in 10 rabbits treated with 0.8 UI/kg is shown in Fig 4A. Insulinemia values were 165 ± 51 and 158 ± 31 μIU/mL, 30 and 60 minutes after insulin administration, respectively. No significant change in MABP, PaO_2, $PaCO_2$, and arterial pH was observed (data not shown).

Six rabbits were "high responders", 4 were "mild responders" (see Materials and Methods for the classification of high and mild responders). The glycemic curves of the 2 groups are shown in Fig 4B. Besides showing significantly different values at all time points, except the 40-minute one, the 2 curves also differ in that in the "high responders" group glycemia continued to decrease up to 60 minutes (the 60-minute value was significantly lower than the 30-minute one), whereas in the "mild responders" group, the glycemia decrease stopped at 30 minutes, because the subsequent time point values did not differ significantly from the 30-minute one. The insulinemia of the "high responders" group never differed significantly from that of the "mild responders" group.

Vitreous glucose concentration of the "high responders" group was significantly lower than that of the "mild responders" group; vitreous glucose of both groups was significantly lower than the control value (Table 3). Retinal [^{3}H]-radioactivity levels and [^{3}H]-[R/VH] of the "high responders" group were significantly higher than both control and "mild responders" group values, while the "mild responders" group values did not differ from those of controls (Table 4). Radioactivity levels in vitreous did not differ from control values neither in "high responders" nor in "mild responders" group (Table 4). In other ocular tissues, radioactivity levels and tissue to vitreous ratios of radioactivity levels of either "high responders " or "mild responders" groups did not differ from control values (data not shown).

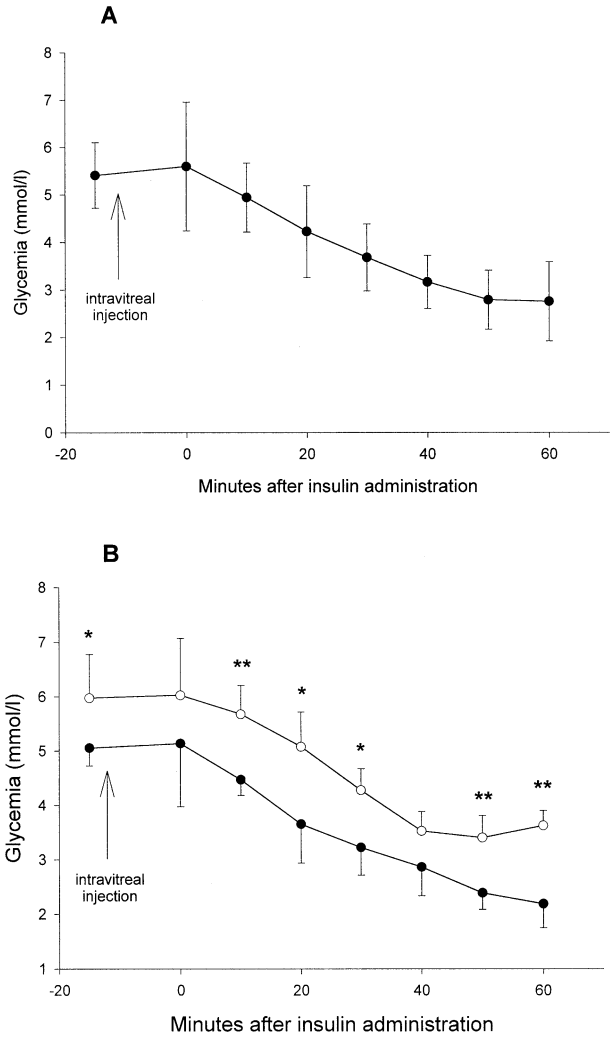

Fig 4. (A) Time-courses of glycemia in 10 rabbits given 0.8 IU/kg subcutaneous insulin (Protocol B) and killed 60 minutes after insulin administration (75 minutes and after the intravitreal injection). Data are means ± SD. (B) Time-courses of glycemia in the "high responders" (○) (6 rabbits) and "mild responders" (●) (4 rabbits,) subgroups of Protocol B insulin-treated rabbits. Data are means ± SD. *P < .05 and **P < .01 *v* "mild responders".

In the vitreous, retina, and choroid extracts of either "high responders" or "mild responders" rabbits, the percentages of 2-DG and 2-DG-6-phosphate did not differ significantly from those of control rabbits (data not shown).

Table 3. Vitreous Glucose Concentration (mmol/L) in Control and Protocol B Insulin-Treated (60 minutes after insulin administration) "High Responders" and "Mild Responders" Groups

Control (8)	"High Responders" (6)	"Mild Responders" (4)
2.88 ± 0.28	2.01 ± 0.20†	2.48 ± 0.24*‡

NOTE. Values are means ± SD. Number of rabbits is shown in parentheses.

*P < .05 and †P < .001 *v* control group, respectively.

‡P < .05 *v* "high responders."

Table 4. [^{3}H]-Radioactivity Levels (dpm/mg or dpm/μL) in Vitreous Humor and Retina and Retina to Vitreous Ratio of [^{3}H]-Radioactivity Levels ([^{3}H]-[R/VH]) in Control and Insulin-Treated (Protocol B) "High Responders" and "Mild Responders" Groups 75 Minutes After the Intravitreal Injection of 9.25 kBq [^{3}H]-2-DG

	Control (5)	"High Responders" (6)	"Mild Responders" (4)
Vitreous humor	175.5 ± 30.7	130.9 ± 33.4	160.1 ± 30.9
Retina	511.8 ± 60.7	705.6 ± 119.8*†	506.6 ± 97.9
[^{3}H]-[R/VH]	3.1 ± 0.3	5.4 ± 1.5*‡	2.9 ± 0.6

NOTE. Values are means ± SD. Number of rabbits is shown in parentheses.
*$P < .01$ *v* control group.
†$P < .05$ and ‡$P < .01$ "mild responders" group, respectively.

[^{14}C]-Radioactivity

In rabbits treated according to both protocols A and B, both the levels of [^{14}C]-radioactivity and the tissue to vitreous humor ratios of radioactivity levels did not differ from control values in all ocular tissues (data not shown). In the rabbits treated with 1.2 IU/kg insulin (Protocol A) the percentage of [^{14}C]-radioactivity in the vitreous due to [^{14}C]-lactate was 25% ± 3% (n = 4) significantly ($P < .05$) higher than in control rabbits.

Hypoglycemia With Maintenance of Vitreous Glucose Levels

The time-course of glycemia in this rabbit group (which received insulin 1.2 IU/kg 15 minutes before intravitreal injection) is shown in Fig 5. In the eyes that had received 1 mg D-glucose, vitreous glucose concentration was equal to that of control rabbits (Table 5). Retinal [^{3}H]-radioactivity levels and [^{3}H]-[R/VH] values were significantly higher than control values, both in the glucose-injected eyes and the contralateral eyes (that had received no exogenous glucose) (Table 5). In vitreous humor (Table 5) and other ocular tissues (data not shown) of both glucose-injected and contralateral eyes, radioactivity levels and tissue to vitreous ratios of radioactivity levels did not differ significantly from control values (data not shown).

No significant difference between control, glucose-injected and contralateral eyes was found for [^{14}C]-radioactivity levels and tissue to vitreous ratios of [^{14}C]-radioactivity levels (data not shown).

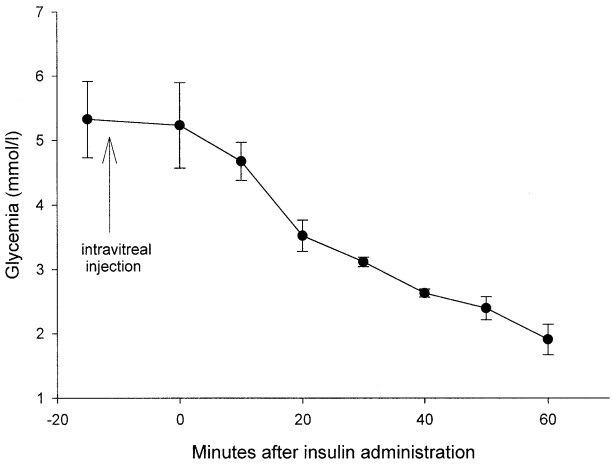

Fig 5. Time-course of glycemia in 4 rabbits treated with subcutaneous insulin (1.2 IU/kg) 15 minutes after injecting intravitreally the labelled compounds plus 1 mg D-glucose in one eye. Data are means ± SD.

Normoglycemic Hyperinsulinemia

Three rabbits were given subcutaneous insulin (1.2 IU/kg) and their glycemia was maintained at basal levels by intravenous glucose infusion (Fig 6); [^{3}H]-2-DG and [^{14}C]-D-glucose were injected intravitreally 60 minutes after insulin administration and the eyes enucleated 75 minutes after the injection. Vitreous glucose and lactate concentrations were not different from control values. No significant change in MABP, PaO_2, $PaCO_2$, and arterial pH was observed. Mean plasma insulin levels were similar to those of Protocol A "high responders" rabbits that received the same insulin dose (Fig 6).

[3H]-Radioactivity

The levels of [^{3}H]-radioactivity in the vitreous, retina, and other ocular tissues in the normoglycemic hyperinsulinemic rabbits did not differ significantly from control values (data not shown). Tissue to vitreous ratios of radioactivity levels did not differ from control values as well.

Normoglycemic hyperinsulinemia did not change the percentages of [^{3}H]-2-DG and [^{3}H]-2-DG-6-phosphate in vitreous humor, retina, and choroid (data not shown).

[^{14}C]-Radioactivity

In the normoglycemic hyperinsulinemic group, both retinal [^{14}C]-radioactivity levels and retina to vitreous ratios of radioactivity levels ([^{14}C]-[R/VH]) were significantly lower than those of the control group (Table 6). Choroid [^{14}C]-radioactivity levels and choroid to vitreous ratios of radioactivity levels were also lower than control values, but the differences did not reach statistical significance ($P = .054$ for radioactivity levels, $P = .097$ for choroid to vitreous ratios). In the vitreous humor and other ocular tissues, radioactivity levels and tissue to vitreous ratios of radioactivity levels did not differ from control values (data not shown). Normoglycemic hyperinsulinemia did not change nor the percentages of [^{14}C]-D-glucose and [^{14}C]-lactate in vitreous humor, retina, and choroid (data not shown).

Insulin Levels in the Vitreous Humor

Vitreous immunoreactive insulin levels in control rabbits were 9 ± 4 μIU/mL. In no insulin-treated rabbit group did insulin levels increase above control values (data not shown).

Table 5. [^{3}H]-Radioactivity Levels in Vitreous Humor and Retina and Retina to Vitreous Ratio of [^{3}H]-Radioactivity Levels ([^{3}H]-[R/VH]) in Control Eyes, Hypoglycemic Rabbit Eyes That Also Received 1 mg Glucose Intravitreally, Contralateral Eyes (receiving no intravitreal glucose)

	Control	Intravitreal Glucose	Contralateral
Vitreous humor	175.5 ± 30.7 (5)	125.2 ± 48.4 (4)	128.1 ± 24.4 (4)
Retina	511.8 ± 60.7 (5)	628.7 ± 63.6* (4)	711.1 ± 101.0† (4)
[^{3}H]-[R/VH]	3.1 ± 0.3 (5)	5.4 ± 2.3* (4)	6.0 ± 0.7* (4)
Vitreous glucose (mmol/L)	2.88 ± 0.28‡ (8)	2.79 ± 0.37‡ (4)	2.01 ± 0.23 (4)

NOTE. Glucose vitreous concentrations are also shown. Values are means ± SD. Number of samples is shown in parentheses.
*$P < .05$ and †$P < .01$ *v* control group, respectively.
‡$P < .01$ *v* contralateral eyes.

DISCUSSION

Glucose is the principal metabolic substrate for the retina in mammals,[1-3] being essential for maintaining the functional activity of the retina[3,4]; it can be supplied to the tissue by both vitreous humor and blood.[10,28] Yet, the impact of hypoglycemia on retinal glucose metabolism has been poorly investigated. In this study, we showed that insulin-induced hypoglycemia selectively increased the 2-DG uptake rate in the retina. Normoglycemic hyperinsulinemia had no effect on 2-DG uptake, but apparently caused a reduction of retinal lactate.

The rabbit retina takes up intravitreally injected 2-DG, the uptake by the retina being the dominant route of the clearance of vitreal 2-DG.[10] Inside the cells, 2-DG can be phosphorylated by hexokinase to 2-DG-6-phosphate, which is not noticeably metabolized further[24] and is trapped within cells. 2-DG-6-phosphate is also a good substrate for glucose-6-phosphatase,[25] but glucose-6-phosphatase activity in the retina is low.[26,27]

In agreement with the findings of Berkowitz et al,[10] we found that after intravitreal injection of [^{3}H]-2-DG, [^{3}H]-radioactivity levels in the retina were higher, from 25 minutes onward, than those in the vitreous humor in control, untreated rabbits. This indicated that [^{3}H]-2-DG was taken up and phosphorylated by retinal cells. [^{3}H]-2-DG-6-phoshate accounted for 47% of total retinal [^{3}H]-radioactivity, 75 minutes after the intravitreal injection. The decrease of retinal [^{3}H]-radioactivity levels from 45 minutes onward (see Fig 1) is probably due to the diffusion out of the retinal cells of unphosphorylated 2-DG.[11] Retinal [^{14}C]-radioactivity was entirely due to [^{14}C]-lactic acid, thus showing that intravitreally injected [^{14}C]-D-glucose was taken up and metabolized to [^{14}C]-lactic acid by the retina.

We found that after injecting intravitreally [^{3}H]-2-DG to rabbits with established hypoglycemia (Protocol A), retinal [^{3}H]-radioactivity levels and [^{3}H]-[R/VH] were markedly higher than control values.

Because vitreous glucose level of hypoglycemic rabbits was reduced by about 50%, in these rabbits the vitreal [^{3}H]-2-DG to endogenous glucose ratio became gradually higher than the control value; theoretically, this might have contributed to observed increases of the radioactivity level and [^{3}H]-[R/VH], because D-glucose might act as a competitive inhibitor of 2-DG uptake.[22] Yet, in the eyes of hypoglycemic rabbits, which also received intravitreally 1 mg D-glucose in addition to the labeled compounds ("hypoglycemia with maintenance of vitreous glucose levels" experiment), retinal [^{3}H]-radioactivity levels and [^{3}H]-[R/VH] values were also significantly higher than the control value, even though in these eyes the vitreal [^{3}H]-2-DG to glucose ratio was never higher than in control eyes because vitreous glucose level was initially higher than the control value and equalled it at the end of the experiment. We also performed experiments according to Protocol A, but injecting a halved amount of [^{3}H]-2-DG, ie, 4.625 kBq. In these eyes, therefore, the vitreal [^{3}H]-2-DG to glucose was initially lower than the ratio value of control rabbits injected with 9.250 kBq [^{3}H]-2-DG and gradually approached to that value as vitreal glucose decreased. The results of these experiments showed that hypoglycemic rabbit eyes injected with 4.625 kBq [^{3}H]-2-DG showed a [^{3}H]-[R/VH] value also higher than that of

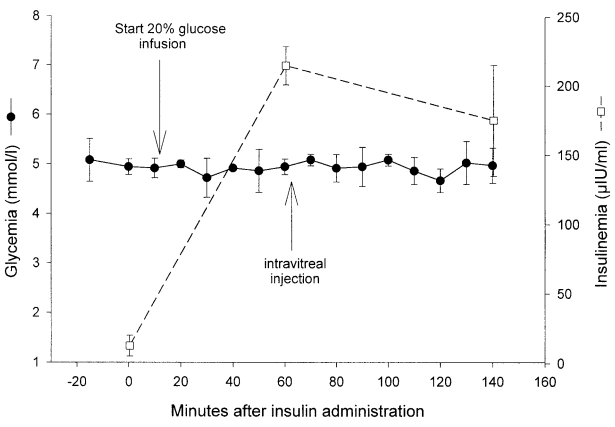

Fig 6. Glycemia (●) and insulinemia (□) during normoglycemic clamp experiments (3 rabbits). Data are means ± SD.

Table 6. Values of [^{14}C]-Radioactivity Levels (dpm/mg) in the Retina and Choroid and Retina to Vitreous ([^{14}C]-[R/VH]) and Choroid to Vitreous ([^{14}C]-[Ch/VH]) Ratios of [^{14}C]-Radioactivity Levels in Control and Normoglycemic Hyperinsulinemic Groups 75 Minutes After the Intravitreal Injection of 37.0 kBq of ^{14}C-D-Glucose

	Control (5)	Normoglycemic Hyperinsulinemic (3)
Retina	581.4 ± 71.7	460.0 ± 57.2*
[^{14}C]-[R/VH]	1.14 ± 0.11	0.84 ± 0.20*
Choroid	268.6 ± 46.6	200.3 ± 16.2
[^{14}C]-[Ch/VH]	0.52 ± 0.12	0.37 ± 0.05

NOTE. Values are means ± SD. Number of rabbits is shown in parentheses.
*$P < .05$ *v* control group.

control rabbit eyes receiving 9.250 kBq [^{3}H]-2-DG (see Fig 3). It can therefore be concluded that the decrease of vitreal endogenous glucose and the resultant increase of the vitreal [^{3}H]-2-DG to glucose ratio, did not contribute substantially to the observed increases of retinal [^{3}H]-radioactivity levels and [^{3}H]-[R/VH].

Because the rabbit retina can use both blood-derived and vitreous-derived glucose,[10,28] it might be thought that hypoglycemia-induced decrease of blood to retina glucose transfer rate may cause an increase of the rate of uptake and consumption of vitreous glucose (and 2-DG) (during hypoglycemia, the passage rate across the blood-retinal barrier decreases because glucose crosses the barrier by passive facilitated diffusion.[29-31]). Yet, this could happen only if blood-derived and vitreous-derived glucose both contributed to extracellular glucose level in the retina; under this condition, a reduction of blood to retina glucose transfer rate would decrease the extracellular retinal glucose concentration, thus causing an increase of the gradient between vitreous and extracellular retinal space, which in turn would increase the diffusion rate from the vitreous. This would result in an increase of the rate of the uptake of vitreous glucose and 2-DG. Yet, Adler and Southwick[32] have shown that the glucose concentration in the interphotoreceptor matrix adjacent to the retina is very low (below 0.01 mmol/L, the limit of detection, in the rabbit retina), being much higher in the interphotoreceptor matrix adjacent to the retinal pigment epithelium (1.6 mmol/L); these data show that virtually all blood-derived glucose is taken up by the photoreceptor layer (the outermost retinal layer) and, therefore, does not reach the inner layers, which should be supplied by vitreous-derived glucose. Thus, the decrease of blood to retina glucose transfer rate should not determine any significant increase of the vitreous to extracellular retinal space glucose concentration gradient and, therefore, should not augment the vitreous to retina diffusion rate. In addition, we found that in the Protocol B "mild responders" group a decrease of the glucose transport rate across the blood-retinal barrier occurred, as indicated by the decrease of the vitreous glucose concentration, but it was not linked to any increase of retinal [^{3}H]-radioactivity levels or [^{3}H]-[R/VH]. Hence, the decrease of glucose transport rate across the blood-retinal barrier should not increase the rate of uptake and consumption of vitreous glucose and should not therefore contribute to the observed increases of retinal [^{3}H]-radioactivity levels and [^{3}H]-[R/VH].

The increases of retinal [^{3}H]-radioactivity levels and [^{3}H]-[R/VH] are not attributable to an increase of the phosporylation rate because we found that hypoglycemia did not augment the percentage of total retinal [^{3}H]-radioactivity due to [^{3}H]-2-DG-6-phosphate.

Thus, the increases of retinal [^{3}H]-radioactivity [^{3}H]-[R/VH] appear to indicate that hypoglycemia increases the uptake rate of glucose by the retina.

The increase of the vitreal [^{14}C]-lactate percentage concentration found in hypoglycemic rabbits (Protocol A) indicates an increased conversion of vitreal [^{14}C]-D-glucose to [^{14}C]-lactate. Because lactate appears to be produced mainly by the inner retina,[4,33] which has a high concentration of glycolytic enzymes,[34] our finding may suggest that hypoglycemia induces an increase of glucose uptake also in the inner retina.

The stimulation of retinal glucose uptake by hypoglycemia may be attributed to factor(s) released to counteract the effects of hypoglycemia. Adenosine triphosphate (ATP) can be released by retinal pigment epithelium cells and can be degraded to adenosine by ectoenzymes.[35] ATP can also be released by Muller cells.[36] Both ATP[37] and adenosine[38,39] are able to stimulate glucose uptake by facilitative glucose transporters and adenosine, and purinergic receptors are expressed in various retinal cellular types.[40-42] Hence, local release of adenosine and/or ATP may be responsible for the observed increase of retinal glucose, which may counteract the effects of hypoglycemia. Accordingly, it has been shown that acute hypoglycemia causes a compensatory increase in retinal blood flow in pigs, which is mediated by adenosine.[43] Adenosine also promotes the hydrolysis of glycogen retinal stores,[44] which can also be regarded as a compensatory response to hypoglycemia. Acting at A2 receptors (and via activation of protein kinase A), adenosine may also acutely increase the functional expression in the plasma membrane of a SGLT-like Na^+/glucose cotransporter[45] in the retina. The results of the in vitro study by Vilchis and Salceda[11] provide evidence that retinal glucose transport is also mediated by a Na^+/glucose cotransporter, because these investigators found that most of the in vitro 2-DG uptake by the rat retina was both sodium-dependent, being inhibited by 90% in sodium-free medium, and energy-dependent, being inhibited 75% by iodoacetate. They also found that 2-DG uptake was only partially inhibited by the facilitative glucose transporters inhibitors, phloretin and cytochalasin B. Thus, in addition facilitated-diffusion GLUT transporters,[46-48] a Na^+/glucose cotransporter appears to be operating in the retina. The activity of this cotransporter may also be increased by hypoglycemia directly, ie, through the decrease of the retinal extracellular glucose concentration. Nishizaki and Matsuoka[49] have found that facilitated-diffusion GLUT transporters (GLUT1) and a SGLT-like Na^+/glucose cotransporter are coexpressed in brain artery endothelial cells and that acute (1 hour) exposure to low glucose concentration enhances the activity of the SGLT-like Na^+/glucose cotransporter, leading to an increase of 2-DG uptake by the SGLT-like glucose transporter. The enhancement of the cotransporter activity appears to be mediated by decrease of cytosolic glucose. SGLT1 Na^+-glucose cotransporter is also expressed in brain neurons; it appears to be inactive under normal conditions, being activated during metabolic stress, when the interstitial glucose concentration may decrease far below the Km value of GLUT3.[50] Hypoglycemia may therefore increase the retinal cotransporter activity by causing a decrease of the retinal extracellular glucose concentration.

The hypoglycemia-induced increase of sympathetic outflow and norepinephrine release[51,52] may also contribute to the 2-DG uptake stimulation. Norepinephrine, which has been found in the retina within the sympathetic neurons innervating the eye from the superior cervical ganglion,[53] is known to stimulate 2-DG uptake by astrocytes.[54,55] This effect is mediated by arachidonic acid.[54] which can be released by norepinephrine via activation of α- or β-adrenergic receptors,[56-59] which are both present in the retina.[60]

Protocol B experiments show that the increase of retinal 2-DG uptake can occur also after a brief period (10 to 20

minutes) of moderate hypoglycemia. In these experiments, retinal [^{3}H]-radioactivity levels and [^{3}H]-[R/VH] only increased above control values in the "high responder" rabbits, whose glycemia was lower than 2.8 mmol/L for at least 2 consecutive plasma glucose determinations (ie, for at least 10 minutes), while in the "mild responders" group retinal [^{3}H]-radioactivity levels and [^{3}H]-[R/VH] values were the same as in control rabbits. Hence, a threshold value of glucose levels may exist, below which the mechanism responsible for the increase of retinal [^{3}H]-2-DG uptake rate is activated.

The results of normoglycemic hyperinsulinemic rabbits showed that the increase of plasma insulin levels did not contribute to the stimulation of 2-DG caused by insulin-induced hypoglycemia. This finding contrasts with stimulation of retinal 2-DG uptake elicited by insulin in the rat retina in vitro[11]; possible explanations are the limited ability of insulin to cross the blood-retinal barrier[19] and the possibly altered retinal sensitivity in the in vitro incubation medium.[61,62] Normoglycemic hyperinsulinemia decreased [^{14}C]-radioactivity levels and [^{14}C]-tissue to vitreous ratios of radioactivity levels in the retina. Because [^{14}C]-lactic acid totally accounts for the retinal [^{14}C]-radioactivity, this finding suggests that normoglycemic hyperinsulinemia may reduce lactate retinal level, possibly increasing its removal from the retina. The removal increase might be ascribed to an increase of uveal blood flow; insulin increases blood flow in some districts[63] and causes dilatation of retinal arterioles in vitro.[64] It is also possible that insulin stimulates the outward transport across the blood retinal barrier as the hormone stimulates the uptake of various substrates by cultured retinal pigment epithelium cells,[65-67] and insulin receptors have been found in these cells.[15,68] In addition, in vivo experiments have suggested that insulin may increase the outward transport across the blood retinal barrier of intravitreally injected fluorescein.[69]

REFERENCES

1. Futterman S, Kinoshita J: Metabolism of the retina. I. Respiration of cattle retina. J Biol Chem 234:723-726, 1959
2. Cohen HL, Noell KW: Glucose catabolism of rabbit retina before and after development of visual function. J Neurochem 5:253-276, 1960
3. Winkler BS: Glycolytic and oxidative metabolism in relation to retinal function. J Gen Physiol 77:667-692, 1981
4. Ames A, Li Y, Heher CE, et al: Energy metabolism of rabbit retina as related to function: High cost of Na^+ transport. J Neurosci 12:840-853, 1992
5. Skandries W, Heinrich H: Differential effects of mild hypoglycemia on proximal and distal structures in man as revealed by electroretinography. Neurosci Lett 134:165-168, 1992
6. Lansel N, Niemeyer G: Effects of insulin under normal and low glucose on retinal electrophysiology in the perfused cat eye. Invest Ophthalmol Vis Sci 38:792-799, 1997
7. Niemeyer G: Glucose concentration and retinal function. Clin Neurosci 4:327-335, 1997
8. Weiss H: The carbohydrate reserve in the vitreous body and retina of the rabbit's eye during and after pressure ischaemia and insulin hypoglycemia. Ophthalmic Res 3:360-371, 1972
9. Bito LZ, Salvador EV, Petrinovic L: Intraocular fluid dynamics. IV. Intraocular sites of solute utilisation and transport as revealed by studies on aphakic eyes. Exp Eye Res 26:47-55, 1978
10. Berkowitz AB, Garner HM, Wilson AC, et al: Non-destructive measurement of retinal glucose transport and consumption in vivo using NMR spectroscopy. J Neurochem 64:2325-2331, 1995
11. Vilchis C, Salceda RCharacterization of [2-^{3}H]deoxy-D-glucose uptake in retina and retinal pigment epithelium of normal and diabetic rats. Neurochem Int 28:213-219, 1996
12. Waldbillig RJ, Fletcher RT, Chader GJ, et al: Retinal insulin receptors I: structural heterogeneity and functional characterization. Exp Eye Res 45:823-835, 1987
13. Rosenzweig SA, Zetterstrom C, Benjamin A: Identification of retinal insulin receptors using site-specific antibodies to a carboxyl-terminal peptid of the human insulin receptor α-subunit: Up regulation of neuronal insulin receptors in diabetes. J Biol Chem 265:18030-18034, 1990
14. Cotlier E, Davidson C: Insulin receptors in calf and human retinal blood vessels. Ophthalmic Res 15:29-37, 1983
15. Waldbillig RJ, Arnold DR, Fletcher RT, et al: Insulin and IGF-I binding in developing chick neural retina and pigment epithelium: A characterization of binding and structural differences. Exp Eye Res 53:13-22, 1991
16. Sinha AC, Stuart CA, Srivastava SK: Receptors for insulin and IGF-I in bovine lens epithelium. Lens Eye Toxic Res 7:133-141, 1990
17. Jacobs DB, Ireland M, Pickett T, et al: Functional characterization of insulin and IGF-I receptors in chicken lens epithelial and fiber cells. Curr Eye Res 11:1137-1145, 1992
18. Naeser P: Insulin receptors in human ocular tissues. Immunohistochemical demonstration in normal and diabetic eyes. Ups J Med Sci 102:35-40, 1997
19. Shires TK, Braddock KJ, Pulido JS: Insulin in the vitreous of the normal and streptozotocin-induced diabetic rat. Peptides 13:671-675, 1992
20. Boyle PJ, Schwartz NS, Shah SD, et al: Plasma glucose concentrations at the onset of hypoglycemic symptoms in patients with poorly controlled diabetes and in nondiabetics. N Engl J Med 318: 1487-492, 1988
21. Bryan RM Jr, Eichler MY, Johnson TD, et al: Cerebral blood flow, plasma catecholamines, and electroencephalogram during hypoglycemia and recovery after glucose infusion. J Neurosurg Anesthesiol 6:24-34, 1994
22. Kletzien RF, Perdue JF: Sugar transport in chick embryo fibroblasts. J Biol Chem 249:3366-3374, 1974
23. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: A method for quantifying insulin secretion and resistence. Am J Physiol 237:E214-E223, 1979
24. Wohlhueter RM, Plagemann PGW: The roles of transport and phosporylation in nutrient uptake in cultured animal cells. Int Rev Cytol 64:171-240, 1980
25. Pessine JE, Bell GI: Mammalian facilitative glucose transporter family: Structure and molecular regulation. Annu Rev Physiol 54:911-930, 1992
26. Blair NP, Shaw WE, Yue B: Glucose-6-phosphatase activity in the retina of the awake rat. Invest Ophthalmol Vis Sci 30:2268-2271, 1989
27. Poitry-Yamate C, Tsacopoulos MGlial (Muller) cells take up and phosphorylate [3H]2-deoxy-D-glucose in mammalian retina. Neurosci Lett 122:241-244, 1991
28. Wang L, Bill A: Effects of constant and flickering light on retinal metabolism in rabbits. Acta Ophthalmol Scand 75:227-231, 1997
29. DiMattio J, Zadunaisky JA: Glucose transport into the ocular compartments of the rat. Exp Eye Res 32:517-532, 1981

30. Ennis SR, Johnson JE, Pautler EL: In situ kinetics of glucose transport across the blood-retinal barrier in normal rats and rats with streptozocin-induced diabetes. Invest Ophthalmol Vis Sci 23:447-456, 1982

31. To CH, Hodson SA: The glucose transport in retinal pigment epithelium is via passive facilitated diffusion. Comp Biochem Physiol A Mol Integr Physiol 121:441-444, 1998

32. Adler AJ, Southwick RE: Distribution of glucose and lactate in the interphotoreceptor matrix. Ophtalmic Res 24:243-252, 1992

33. Padnick-Silver L, Linsenmeier RA: Quantification of in vivo anaerobic metabolism in the normal cat retina through intraretinal pH measurements. Vis Neurosci 19:793-806, 2002

34. Lowry OH, Roberts NR, Lewis C: The quantitative histochemistry of the retina. J Biol Chem 220:879-892, 1956

35. Mitchell CH: Release of ATP by a human retinal pigment epithelial cell line: Potential for autocrine stimulation through subretinal space. J Physiol 534:193-202, 2001

36. Newman EA: Glial cell inhibition of neurons by release of ATP. J Neurosci 23:1659-1666, 2003

37. Kim MS, Lee J, Ha J, et al: ATP stimulates glucose transport through activation of P2 purinergic receptors in C(2)C(12) skeletal muscle cells. Arch Biochem Biophys 401:205-214, 2002

38. Angello DA, Berne RM, Coddington NM: Adenosine and insulin mediate glucose uptake in normoxic rat hearts by different mechanisms. Am J Physiol 265:H880-885, 1993

39. Daval JL, Nicolas F: Non-selective effects of adenosine A1 receptor ligands on energy metabolism and macromolecular biosynthesis in cultured central neurons. Biochem Pharmacol 55:141-149, 1998

40. Li B, Rosenbaum PS, Jennings NM, et al: Differing roles of adenosine receptor subtypes in retinal ischemia-reperfusion injury in the rat. Exp Eye Res 68:9-17, 1999

41. Reifel Saltzberg JM, Garvey KA, Keirstead SA: Pharmacological characterization of P2Y receptor subtypes on isolated tiger salamander Muller cells. Glia 42:149-159, 2003

42. Ishii K, Kaneda M, Li H: Neuron-specific distribution of P2X7 purinergic receptors in the monkey retina. J Comp Neurol 459:267-77, 2003

43. Zhu Y, Gidday JM: Hypoglycemic hyperemia in retina of newborn pigs. Involvement of adenosine. Invest Ophthalmol Vis Sci 37: 86-92, 1996

44. Osborne NN: [3H]glycogen hydrolysis elicited by adenosine in rabbit retina: Involvement of A_2-receptors. Neurochem Int 14:419-422, 1989

45. Hirsch JR, Loo DD, Wright EM: Regulation of Na+/glucose cotransporter expression by kinases in Xenopus laevis oocytes. J Biol Chem 271:14740-14746, 1996

46. Kumagai AK, Glasgow BJ, Pardridge WM: GLUT1 glucose transporter expression in the diabetic and nondiabetic human eye. Invest Ophthalmol Vis Sci 35:2887-2894, 1994

47. Watanabe T, Mio Y, Hoshino FB, et al: GLUT2 expression in the rat retina: Localization at the apical ends of Muller cells. Brain Res 655:128-134, 1994

48. Watanabe T, Matsushima S, Okazaki M, et al: Localization and ontogeny of GLUT3 expression in the rat retina. Brain Res Dev Brain Res 94:60-66, 1996

49. Nishizaki T, Matsuoka T: Low glucose enhances Na+/glucose transport in bovine brain artery endothelial cells. Stroke 29:844-849, 1998

50. Poppe R, Karbach U, Gambaryan S, et al: Expression of the Na+-D-glucose cotransporter SGLT1 in neurons. J Neurochem 69:84-94, 1997

51. Fagius J, Niklasson F, Berne C: Sympathetic outflow in human muscle nerves increases during hypoglycemia. Diabetes 35:1142-1149, 1986

52. Fagius J, Berne CChanges of sympathetic nerve activity induced by 2-deoxy-D-glucose infusion in humans. Am J Physiol 256:E714-720, 1989

53. Hadjiconstantinou M, Cohen J, Neff NH: Epinephrine: A potential neurotransmitter in retina. J Neurochem 41:1440-1444, 1983

54. Yu N, Martin JL, Stella N, et al: Arachidonic acid stimulates glucose uptake in cerebral cortical astrocytes. Proc Natl Acad Sci USA 90:4042-4046, 1993

55. Pellerin L, Stolz M, Sorg O, et al: Regulation of energy metabolism by neurotransmitters in astrocytes in primary culture and in an immortalized cell line. Glia 21:74-83, 1997

56. Malik KU: Interaction of arachidonic acid metabolites and adrenergic nervous system. Am J Med Sci 295:280-286, 1988

57. Ruan Y, Kan H, Parmentier JH, et al: Alpha-1A adrenergic receptor stimulation with phenylephrine promotes arachidonic acid release by activation of phospholipase D in rat-1 fibroblasts: Inhibition by protein kinase A. J Pharmacol Exp Ther 284:576-585, 1998

58. Shaffer JE, Malik KU: Enhancement of prostaglandin output during activation of beta-1 adrenoceptors in the isolated rabbit heart. J Pharmacol Exp Ther 223:729-735, 1982

59. Lew DB, Nadel GL, Malik KU: Prostaglandin E2 synthesis elicited by adrenergic stimuli in guinea pig trachea is mediated primarily via activation of beta 2 adrenergic receptors. Prostaglandins 44:399-412, 1992

60. Osborne NN: Neuromediators and their receptors (adrenergic and endothelin types) in the eye. Therapie 48:549-558, 1993

61. Ames A, Nesbett FB: In vitro retina as an experimental model of the central nervous system. J Neurochem 37:867-877, 1981

62. Winkler BS: Buffer dependence of retinal glycolysis end ERG potentials. Exp Eye Res 42:585-593, 1986

63. Cleland SJ, Petrie JR, Ueda S, et al: Insulin as a vascular hormone: Implications for the pathophysiology of cardiovascular disease. Clin Exp Pharmacol Physiol 25:175-184, 1998

64. Su EN, Yu DY, Alder VA, et al: Direct vasodilatatory effect of insulin on isolated retinal arterioles. Invest Ophthalmol Vis Sci 37: 2634-2644, 1996

65. Miceli MV, Newsome DA: Cultured retinal pigment epithelium cells from donors with type I diabetes show an altered insulin response. Invest Ophthalmol Vis Sci 32:2847-2853, 1991

66. Miceli MV, Newsome DA: Insulin stimulation of retinal outer segment uptake by cultured human retinal pigment epithelial cells determined by a flow cytometric method. Exp Eye Res 59:271-280, 1994

67. Budohoski L, Opas M: Effects of insulin and a tumor promoter, TPA, on glucose transport and metabolism in retinal pigmented epithelium in vitro. Biochem Int 27:777-782, 1992

68. Takagi H, Yoshimura N, Tanihara H, et al: Insulin-like growth factor-related genes, receptors, and binding proteins in cultured human retinal pigment epithelial cells. Invest Ophthalmol Vis Sci 35:916-923, 1994

69. Jones CW, Cunha-Vaz JG, Rusin MM: Vitreous fluorophotometry in the alloxan- and streptozotocin-treated rat. Arch Ophthalmol 100:1141-1145, 1982