Effect of PKCβ on Retinal Oxygenation Response in Experimental Diabetes

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Purpose. To test the hypotheses that, in mice, breathing carbogen (95% O₂:5% CO₂) oxygenates the retina better than breathing 100% oxygen, the superior hemiretinal oxygenation response to carbogen inhalation is subnormal early in diabetes, and diabetes-induced elevation of retinal protein kinase C (PKC)β contributes to this pathophysiology.

Methods. Retinal oxygenation response (ΔPO₂) was measured using functional magnetic resonance imaging (fMRI) and either carbogen or 100% oxygen inhalation challenge in C57BL/6J control (C) mice. Retinal ΔPO₂ during carbogen breathing was also measured in PKCβ knockout (C57BL6-Prkcb1 [KO]), 4-month C57BL/6J diabetic (D), and 4-month diabetic PKCβ KO (D+KO) mice. Retinal PKCβ protein expression was assessed by Western analysis.

Results. In C mice, retinal ΔPO₂ during carbogen breathing was significantly greater (P < 0.05) than during oxygen breathing. In D mice, ΔPO₂ during carbogen breathing was significantly lower than in the superior, but not the inferior, hemiretina and was normal (P > 0.05) in the KO group. In the D+KO mice ΔPO₂ was normal (P > 0.05) only at distances less than 1.5 mm from the optic nerve head. PKCβ expression was elevated in the retina in diabetes (P < 0.05), but was significantly decreased (P < 0.05) in D+KO mice.

Conclusions. The present study confirms that, in the mouse, retinal ΔPO₂ patterns during different inhalation challenges or in the presence of diabetes are similar to what has been reported in rats. Diabetes-induced elevation of PKC appears to contribute only locally to subnormal retinal ΔPO₂. This raises the possibility that PKC inhibition therapy may be only regionally effective in treating retinal pathophysiologic associated with diabetic retinopathy.

Identifying diabetes-related biochemical changes in the retina that are associated with early pathophysiology and subsequent histopathology could pinpoint potential targets for therapeutic intervention. The activity of protein kinase C (PKC), a key regulator of diverse phenotypic activity, including cell growth and differentiation, is elevated in the retina of diabetic and galactose-fed rodents.1-3 In particular, elevated expression of the PKCβ isofrom has been implicated in decreased steady state retinal perfusion after 2 weeks of diabetes in the rat.4,5 It is not yet known whether increased PKCβ expression regulates functional aspects of retinal circulatory pathophysiologic associated with longer durations of diabetes.

We have developed a novel functional magnetic resonance imaging (fMRI) method for measuring the retinal oxygenation response to a hyperoxic inhalation challenge in the newborn and adult rat, rabbit, cat, and human.6-14 In this technique, hyperoxia-induced increases in vitreous partial oxygen pressure over the room-air value (ΔPO₂) are measured as increased signal intensity. Good agreement is found between the MRI-measured response and that derived from oxygen electrode data in normal rat retina under similar conditions.12,15 In control rats, during a carbogen challenge, oxygen from the choroid is mostly consumed by the inner plexiform layer and does not enter the preretinal vitreous space to a substantial degree.12,14 A number of interrelated hemodynamic parameters can modulate oxygen supply during a carbogen challenge, including retinal vessel autoregulation, perfusion reserve, ocular perfusion pressure, plasma pH, and vascular density. In normal adult and newborn rats, carbogen breathing oxygenates the retina significantly better than pure oxygen breathing.13 Carbogen, a gas mixture of carbon dioxide (5%) and oxygen (95%), is used clinically instead of 100% oxygen to minimize the vasoconstrictive effects of pure O₂ on retinal blood flow and oxygenation.

Application of this functional MRI method in rat models of diabetic retinopathy has revealed that the superior hemiretina exhibits a lesser (i.e., subnormal) oxygenation response than is found in control (i.e., normal) animals and that this abnormality appears several months before retinal histopathology becomes apparent.7,15 In principle, during a carbogen challenge, reduced oxygen supplied by the retinal circulation and/or increased retinal oxygen consumption could produce a subnormal response. However, a subnormal oxygenation response does not appear to represent retinal hypoxia.16 Because retinal oxygen demand may decrease in experimental diabetes,17,18 our working hypothesis is that measurement of a subnormal retinal ΔPO₂ is primarily a measure of a decrease in oxygen supplied by the retinal circulation.6-8,15

Aminoguanidine (AMG), a compound shown to inhibit retinal PKC activity in diabetes, preserved the retinal oxygenation response at the 3-month time point and prevented the development of retinal histopathology at 15 months of diabetes in rats.2,15,19 In contrast, AMG treatment of galactose-fed rats did not protect the retinal oxygenation response at 3 months, nor did it prevent the development of retinal lesions at 15 months.15,19,20 Galactose-fed rats treated with an aldose reductase inhibitor also preserved the retinal oxygenation response and prevented development of retinal lesions.15 Taken together, these data underscore the use of retinal ΔPO₂ measurements as a potential surrogate marker of the efficacy of treatment in diabetic retinopathy.

In this study, we examined whether in the mouse retinal ΔPO₂ patterns during breathing of carbogen and oxygen as well

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as in diabetes were similar to those in the rat.7,13–15 In addition, we began to test the hypothesis that diabetes-induced elevation of PKCβ in the retina contributes to the development of an early subnormal ΔPO₂. Retinal ΔPO₂ was compared in control, diabetic, and diabetic PKCβ KO mice. Diabetic mice exhibited early biochemical changes, including elevation of PKC activity, similar to those in diabetic rats.5 Furthermore, in long-term (>15 months) experimental diabetes in mice, retinal histopathology (acellular capillaries and pericyte ghosts) developed that is similar to that observed in lesions in long-term diabetes in rats and early in the course of diabetes in humans.21–25

**Methods**

The animals were treated in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Animal Models**

Control (C57BL/6j, C group) mice were purchased from Jackson Laboratories (Bar Harbor, ME) and PKC mice with targeted disruption of the PKCβ gene (KO group) with a C57] background (C57BL6-Pkcb1) were provided by one of the authors (ML).24 The KO mice have a similar lifespan as the C57BL/6j (Letiges M, personal communication, December 2000).

Diabetes was induced in the C57BL/6j and KO mice (starting weight, 20–24 g) by streptozotocin (65 mg/kg; 50 mM citrate buffer [pH 4.5]) intraperitoneal injection once a day for 5 days. Body weight, blood glucose, and urine ketones levels were monitored weekly. Insulin (neutral protamine Hagedorn [NPH]) was administered to allow slow weight gain while maintaining hyperglycemia (blood glucose levels above 400 mg/dL). Normal rodent chow and water were provided ad libitum. After 4 months of diabetes, only those mice with fasting blood glucose levels higher than 400 mg/dL (D group) were studied by functional MRI.

**Western Blot Analysis**

Retinal tissue was homogenized in 30 mM Tris-HCl buffer containing 10 mM EGTA, 5 mM EDTA, 1% Triton X-100, and 250 mM sucrose containing 1 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 15 μg/mL aprotinin, 5 μg/mL leupeptin, 5 μg/mL pepstatin, and 1 mM Na₃VO₄. The homogenate was centrifuged at 5000 × g) was separated on a 10% reducing polyacrylamide gel and then transferred to nitrocellulose membranes. The membranes were blocked in the wash buffer (10 mM Tris-HCl [pH 7.5], 100 mM NaCl and 0.1% Tween 20), containing 5% milk, followed by the incubation with a polyclonal antibody against PKCβ (catalog no. sc-210; Santa Cruz Biotechnology, Santa Cruz, CA) at a dilution of 1:1000. The membranes were washed three times (10 minutes each), and incubated for 1 hour at room temperature with horseradish-peroxidase-linked anti-rabbit Ig (Amersham Pharmacia Biotech, Amersham, UK; 1:2500 dilution), and developed with a Western blot kit (ECL-Plus; Amersham Pharmacia Biotech).

To ensure equal loading among lanes, the expression of the intrinsic protein β-actin was quantified in each lane. The intensities of PKCβII band and β-actin band in each lane were quantified with an automated gel digitizing system (Un-Scan-It; Silk Scientific, Orem, UT).

**Functional MRI Examination**

On the day of the experiment, anesthesia was induced by a single intraperitoneal injection of urethane (36% solution, 0.083 mL/20 g animal weight, prepared fresh daily; Aldrich, Milwaukee, WI). Each mouse was gently positioned on an MRI-compatible custom-made holder with its nose placed in a plastic nose cone. Animals were allowed to breathe spontaneously during the experiment. To maintain the core temperature, a recirculating heated water blanket was used. Rectal temperature was continuously monitored while the animal was inside the magnet.15

MRI data were acquired on a 4.7-T system using a two-turn transmit-and-receive surface coil (1.0 cm diameter) placed over the eye, as previously described.9 (repetition time [TR], 1 second; echo time [TE], 22.7 ms; number of acquisitions [NA], 1; matrix size, 128 × 256; slice thickness, 1 mm; field of view, 18 × 18 mm; sweep width, 25,000 Hz; 2 minutes per image). Examining the initial retinal oxygenation response to a hyperoxic challenge requires careful attention to experimental timing and makes high demands on the animals’ physiology, compared with steady state measurements.

The functional MRI data were collected as follows. Sagittal localize images were first collected and used to position a single 1-mm-thick transverse slice through the center of the eye. The 1-mm slice thickness was needed to obtain an adequate signal-to-noise ratio in a 2-minute image. This slice thickness resulted in some partial volume averaging, so that the final image contained superior and inferior hemiretina, with a relatively minor contribution from the temporal and nasal hemiretina. A capillary tube (1.5-mm inner diameter) filled with distilled water was used as the external standard.

It is important to note that steady state (room air) vitreous oxygen tension cannot be measured by this method, because many factors affect the preretinal vitreous water signal and its relaxation properties. In other words, simply obtaining an image of the eye during room air breathing alone cannot be used to measure retinal oxygenation. In addition, detailed inferences regarding oxygen consumption and delivery in individual retinal layers cannot be made, because functional MRI measures vitreal oxygenation and so reflects general trends in intraretinal oxygenation.14 Nonetheless, as discussed in the introduction, retinal ΔPO₂ have shown promise as a biomarker of treatment efficacy in diabetic retinopathy.

In general, ΔPO₂ data were collected approximately 90 minutes after urethane injection to avoid potential errors due to variable time under urethane anesthesia. Three images were acquired while the animal breathed room air and one image during the inhalation of carbogen or 100% oxygen. The hyperoxic gas exposure was started at the end of the third image. Animals were returned to room air for 5 minutes to allow recovery from the inhalation challenge and were removed from the magnet. A second 2-minute inhalation challenge was performed outside the magnet with care taken to avoid altering the spatial relationship between the animal head and nose cone. At exactly 2 minutes, arterial blood from the abdominal aorta was collected as described previously.9 This blood was analyzed for glucose, percentage of glycated hemoglobin, PaO₂, PaCO₂, and pH. However, because the length of time that the blood was stored before the percent glycated hemoglobin assay, the data produced were not reproducible and so are not reported. Note that this second inhalation challenge (outside the magnet) is needed because it is not feasible to obtain routinely an arterial blood sample from inside the magnet (>40 cm away from the magnet opening) from 25 g mice. In all cases, after the blood collection, the mice were killed with an intracardiac potassium chloride injection and eyes enucleated for Western blot analysis.

**Data Analysis**

To be included in this study, the animal must have demonstrated (1) minimal eye movement during the MRI examination, (2) a nongasping
respiratory pattern before the MRI examination, (3) rectal temperature in the range of 35.5 to 36.5°C, and (4) PaO₂ higher than 350 mm Hg and Paco₂ between 46 and 65 mm Hg during the carbogen challenge. Occasionally, the blood gas machine was not able to read a sample (e.g., due to a clot or excessive air in the capillary tube). In this case, the MRI data were also excluded. Between 5 to 20 animals in each of the treatment groups satisfied the fourth inclusion criterion.

To correct for possible subtle shifting of the animals’ position during the experiment (usually for reasons unrelated to the physiology of the animal, such as settling on the gauze packing) a warp affine image coregistration was performed on each animal with software written in-house. After coregistration, the MRI data were transferred to a computer (Power Mac G4; Apple Computer, Cupertino, CA) and analyzed with the software program Image (available by ftp at zippy. nimh.nih.gov/ or at http://rsb.info.nih.gov/nih-image; developed by Wayne Rasband, National Institutes of Health, Bethesda, MD). Images obtained during room air breathing were averaged to improve the signal-to-noise ratio. All pixel signal intensities in the average-room-air image and the 2-minute carbogen image were then normalized to the same mice, adjusted to the intensity of the intrinsic protein β-actin.

Significant differences (P < 0.05) compared with the control.

Statistical Analysis

The physiological parameters (i.e., blood gas values, rectal temperatures, blood glucose data) were normally distributed and are presented as the mean ± SEM. Comparisons were performed with an unpaired t-test. Separate comparisons of retinal ΔPo₂ between the C and D groups and between the KO and D+KO groups were performed with a generalized estimating equation. This method performs a general linear regression analysis using all the pixels in each subject and accounts for the within-subject correlation between adjacent pixels. When multiple comparisons were made, the likelihood of finding a significant impact for all the comparisons by chance alone is P ≤ 0.0167 (0.05/number of comparisons made) or 0.05/3 = 0.0167). When two samples were compared, P ≤ 0.05 was considered significant.

RESULTS

Characterization of Retinal PKCβ Expression

Figure 1 shows Western blots from representative retinal extracts. PKCβ expression was elevated (P < 0.05) by more than twofold in the retina of 4-month D mice compared with C mice. The expression of PKCβ in the retina was decreased by approximately 60% in the KO mice and 50% in D+KO mice, compared with the C group (P < 0.001). However, no difference (P > 0.05) was found in expression between the KO and
D-KO groups. As shown in Figure 1, despite the significant differences in the content of β-actin among various lanes, the content of β-actin was not significantly different.

**Systemic Physiology**

A summary of the mouse blood glucose, core temperature, and blood parameters measured during the 2-minute hyperoxic challenges are presented in Table 1. As expected, the C group breathing carbogen had higher \( P < 0.05 \) PaCO\(_2\) and lower pH levels than mice breathing 100% oxygen. PaO\(_2\) levels were different \( P < 0.05 \) between carbogen and oxygen breathing. We think that the large differences in PaCO\(_2\) and pH between carbogen and oxygen breathing explains the larger increase in retinal \( \Delta \)PaO\(_2\) during carbogen breathing (Fig. 2) better than the small PaO\(_2\) difference (approximately 10%). Compared with C-group mice, all D-group animals had significantly \( P < 0.05 \) elevated blood glucose levels. There were no significant \( P > 0.05 \) differences in plasma glucose levels between the D and D+KO groups. The PaO\(_2\), pH, and core temperature were not significantly \( P > 0.05 \) different among any of the carbogen-breathing mouse groups (Table 1).

**Functional MRI**

We and others have demonstrated in rats that carbogen breathing produces a larger retinal oxygenation response than that produced by 100% oxygen breathing.\(^{12,13}\) In different groups of control mice, we confirmed that 2 minutes of carbogen breathing (panretinal \( \Delta \)PaO\(_2\) = 142 ± 4, \( n = 20 \)) produced a larger \( P < 0.05 \) oxygenation response than during oxygen inhalation (panretinal \( \Delta \)PaO\(_2\) = 78 ± 5, \( n = 6 \); Fig. 2). Further experiments in the present study were performed with carbogen gas to best detect subtle differences in retinal oxygenation between groups.

Figure 3 summarizes the retinal \( \Delta \)PaO\(_2\) data in all groups. No significant \( P > 0.05 \) differences were found between the superior and inferior hemiretinal \( \Delta \)PaO\(_2\) of the control and PKC KO mice (i.e., no hemiretinal asymmetry) or between the inferior hemiretinal \( \Delta \)PaO\(_2\) in all the groups. A significantly \( P < 0.05 \) subnormal average superior hemiretinal \( \Delta \)PaO\(_2\) was found only in the D group.

Figure 4 summarizes the superior hemiretina pixel \( \Delta \)PaO\(_2\) versus distance from the optic nerve head for the C, D, KO, and D+KO groups. No significant differences \( P > 0.05 \) were found between the inferior hemiretinal \( \Delta \)PaO\(_2\) in any of these groups (data not shown). Although the pansuperior hemiretinal \( \Delta \)PaO\(_2\) of the D+KO mice was not subnormal compared with the KO group \( P > 0.05 \), Fig. 3), retinal \( \Delta \)PaO\(_2\) was normal only in a region less than 1.5 mm from the optic nerve head.

**DISCUSSION**

In this study, we confirmed that the pattern of the mouse retinal response to carbogen and 100% oxygen breathing are

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**Table 1.** Summary of Mouse Systemic Physiology (mean ± SEM)

<table>
<thead>
<tr>
<th>Group</th>
<th>Fasting Blood Glucose (mg/dL)</th>
<th>PaO(_2) (mm Hg)</th>
<th>PaCO(_2) (mm Hg)</th>
<th>pH</th>
<th>Core Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C carbogen (( n = 20))</td>
<td>131 ± 13</td>
<td>551 ± 13</td>
<td>59 ± 1</td>
<td>7.19 ± 0.00</td>
<td>35.7 ± 0.1</td>
</tr>
<tr>
<td>C oxygen (( n = 6))</td>
<td>128 ± 19</td>
<td>637 ± 15*</td>
<td>39 ± 1*</td>
<td>7.32 ± 0.02*</td>
<td>36.1 ± 0.2</td>
</tr>
<tr>
<td>4-Mo D carbogen (( n = 13))</td>
<td>520 ± 30*</td>
<td>587 ± 17</td>
<td>55 ± 2</td>
<td>7.25 ± 0.02</td>
<td>35.8 ± 0.1</td>
</tr>
<tr>
<td>KO carbogen (( n = 9))</td>
<td>126 ± 12</td>
<td>569 ± 14</td>
<td>57 ± 2</td>
<td>7.22 ± 0.05</td>
<td>36.4 ± 0.2</td>
</tr>
<tr>
<td>D+KO carbogen (( n = 5))</td>
<td>522 ± 42*</td>
<td>593 ± 8</td>
<td>56 ± 2</td>
<td>7.24 ± 0.01</td>
<td>35.6 ± 0.2</td>
</tr>
</tbody>
</table>

* Significantly different from C carbogen group, \( P \leq 0.05 \).

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**FIGURE 2.** Inferior and superior hemiretinal \( \Delta \)PaO\(_2\) in control mice breathing either carbogen (Carbo) or 100% oxygen (Oxy). Both hemiretinal responses during oxygen breathing were lower than during carbogen breathing \( * P < 0.05 \). The number of animals used to generate these data is listed above each histogram. Error bars, SEM.

**FIGURE 3.** Histograms of (left) inferior and (right) superior hemiretinal \( \Delta \)PaO\(_2\) in the C and D groups. The inferior hemiretinal \( \Delta \)PaO\(_2\)s were not significantly \( P > 0.05 \) different between any groups. A subnormal \( CP < 0.05 \) superior hemiretinal \( \Delta \)PaO\(_2\) was found only in the D group. The number of animals used to generate these data is listed above each histogram. Error bars, SEM.
Figure 4. Plots of the average superior hemiretina pixel ΔPO₂ versus distance from the optic nerve head for combined C and D groups (left) and for the C and D+KO groups (right).

similar to our previous findings in rats. In addition, we measured a pathophysiologic retinal response pattern (subnormal pansuperior hemiretinal ΔPO₂) after 4 months of diabetes in the mouse that was similar to that in the rat with 3 months of experimental diabetes. This pathophysiologic occurs earlier than the reported development of retinal vascular lesions (acellar capillaries and pericyte ghosts appear at later times [>15 months] in experimental diabetes in mice). It is possible that the present retinal oxygenation differences between the C and D groups were the result of systemic variations between these groups. However, no significant differences in core temperature and blood gas parameters were found (Table 1). Although we did not measure other systemic physiologic parameters, such as blood pressure, it seems reasonable to expect that systemic physiologic differences would similarly affect both the inferior and superior hemiretinal oxygenation response. The lack of differences between the inferior hemiretinal ΔPO₂ in the C and D groups does not support this possibility. Based on these considerations, it appears that the inability of the superior hemiretina to oxygenate in the D group is due to local (i.e., retinal) and not systemic factors.

We have not yet identified any mechanisms that can explain why this early pathophysiologic appears in superior but not inferior hemiretina of diabetic rats. In control and KO mice, no superior-inferior hemiretinal ΔPO₂ asymmetry was detected (Fig. 3). One possibility is that early histopathology may preferentially occur in superior hemiretina of diabetic rats. However, to the best of our knowledge, there are no reports of early changes in histology in the superior hemiretina preferentially. However, the relatively small number of lesions in the diabetic retina at more than 15 months prevents finding a statistical answer to this question (Kern T, unpublished data, 2000). Also, superior-inferior differences in choroidal circulation have not been reported, although in the rat, electrode data suggest that oxygen from the choroid is mostly consumed by the photoreceptors and does not enter the preretinal vitreous space. The rodent superior hemiretina is reported to contain a greater number of ganglion cells than the inferior hemiretina and so would be expected to have a relatively greater metabolic demand. We speculate that this greater metabolic demand may make the superior hemiretina more susceptible to a hyperglycemic insult than the inferior hemiretina. Because control and diabetic animals are handled identically throughout the data collection and analysis aspects of the experiment, factors, such as differences in geometry, are similar for both groups of animals. Note that after 15 months of galactose feeding in rats, both superior and inferior hemiretina are subnormal, suggesting that the early superior hemiretinal subnormal response indicates that perhaps the superior region is more susceptible to diabetes-induced damage. The present data in mice, and results from our previous studies in diabetic and galactose-fed rats, highlights a defect in the superior hemiretinal ΔPO₂ as an early marker of diabetes-related histopathology.

Defining the physiology underlying retinal ΔPO₂ remains an area of ongoing research. To help clarify interpretation of altered ΔPO₂, we note first that during steady state (room air breathing) conditions, retinal oxygen demand and supply are matched so that all the oxygen delivered to the retina is consumed and none is dumped into the preretinal vitreous. In this work, retinal ΔPO₂ is normally maintained by appropriate retinal vasoactivity, with a broad range of ocular perfusion pressures and retinal oxygen consumption rates. However, the retinal circulation is not usually at steady state. Instead, retinal oxygen supply and demand are constantly adapt during daily activities. ΔPO₂ is believed to be a measure of how well retinal oxygen supply works. If baseline PO₂ is either higher or lower than normal but ΔPO₂ is normal, the implication is that the circulation will be able to compensate for the baseline abnormality. A subnormal ΔPO₂ (i.e., an inability to match oxygen supply with demand) is expected to have long-term negative consequence for the health of the retina.

In this study, we investigated the contribution of a specific diabetes-induced biochemical abnormality, increased PKCβ activity, to the ΔPO₂ differences between control and D groups. The results of the current work, and the results of others, indicate that retinal PKC expression is elevated before the appearance of retinal histopathology in diabetic and galactose-fed rats and mice (Fig. 1). Increased PKCβ activity can affect retinal circulatory physiology. For example, drug inhibition of PKCβ has been reported to prevent the decreased retinal perfusion measured in the first couple of weeks after conversion to diabetes, attenuate the increase in leukocyte entrapment in the retinal microcirculation during the first 4-week diabetic period in rats, and inhibit preretinal and optic nerve head neovascularization in a nondiabetic branch retinal vein occlusion pig model. As discussed previously, retinal ΔPO₂ is expected to reflect the contributions of a variety of vascular physiological processes, such as vessel autoregulation, that govern the functional retinal oxygenation response during the carbogen challenge. The relationship between decreased steady state perfusion of the retina (as measured using dye dilution methods, for example) and a subnormal functional response to an inhalation provocation has not yet been investigated. We hypothesized that in 4-month D mice, elevated PKCβ contributed to the development of a subnormal ΔPO₂ in experimental diabetic retinopathy.

The results of this study only partially supported this hypothesis. The reported elevated retinal PKCβ activity appears to be important to the development of a subnormal ΔPO₂ in central retina of D mice. To the best of our knowledge there is no literature that maps PKC activity across the mouse retina in normal and KO mice and more work is needed to chart regional PKC in the mouse eye. Our Western blot data show that the protein expression of PKCβ in the retina was decreased by 60% in the KO mice and 50% in the D+KO mice compared with the control mice. This could suggest that the deletion of the gene for PKCβ was incomplete in the retina, or that the polyclonal antibody used for PKCβ was reacting with some other proteins in the retina. However, given that these mice were homozygous for a targeted disruption, the possibility of incomplete PKC knockout in the retina seems unlikely. It
remains to be determined whether or not chronic inhibition of PKCβ in nongenetically altered rodents will correct central retina oxygenation response defects or, more important, prevent long-term retinal histopathology. Nonetheless, these considerations raise the possibility, for the first time, that specific PKCβ inhibition treatment may only be regionally effective in treating retinal pathophysiology associated with diabetic retinopathy.

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