Intraretinal Oxygenation and Oxygen Consumption in the Rabbit during Systemic Hyperoxia

Stephen J. Cringle and Dao-Yi Yu

Purpose. To determine the ability of the retina and choroid in the avascular region of the rabbit retina to regulate intraretinal oxygen levels during periods of acute systemic hyperoxia.

Methods. Oxygen-sensitive microelectrodes were used to measure oxygen tension as a function of depth through the retina and choroid in anesthetized rabbits before and after stepwise incremental increases in inspired oxygen level. The extent of any oxygen increases throughout the retina and choroid was determined, and retinal oxygen consumption was determined by analyzing intraretinal oxygen distribution.

Results. Increases in systemic arterial oxygen in the rabbit resulted in significant increases in oxygen tension in the choroid and in all retinal layers. There was no apparent regulation of choroidal oxygen tension, as seen in the avascular retina of the guinea pig. Neither was there any increase in oxygen consumption within the retina, which has been shown to dampen the extent of raised oxygen levels in the inner retina in other mammals. Consequently, all retinal layers in the avascular area of the rabbit retina were exposed to high oxygen levels during systemic hyperoxia. The magnitude of the oxygen changes in all retinal layers was consistent with that in the preretinal vitreous.

Conclusions. Unlike other mammals studied to date, the rabbit does not possess any regulatory mechanisms for controlling the intraretinal oxygen environment during acute increases in systemic arterial oxygen levels. This may well account for the reported vulnerability of the rabbit retina to toxic damage during extended periods of systemic hyperoxia. (*Invest Ophthalmol Vis Sci.* 2004;45:3223–3228) DOI:10.1167/iovs.03-1364

Many mammals have demonstrated an ability to limit the increase in inner retinal oxygen tension during systemic administration of supplemental oxygen. It has been shown that the increase in inner retinal oxygen tension during systemic hyperoxia is much less than that in the systemic circulation in monkeys,[1,2] pigs,[3] cats,[4–7] guinea pigs,[8] and rats.[9] In the porcine eye, the regulation is apparently so efficient that there is no increase at all in the oxygen level in the innermost retina, remote from the major retinal vessels, despite dramatic increases in choroidal oxygen tension.[3] More recent work in the rat has suggested that the muted response of the inner retina to systemic hyperoxia is due to both regulatory control of oxygen delivery and increased oxygen consumption in the inner retina.[10] In the avascular retina of the guinea pig, there is obviously no scope for regulation of retinal blood flow to account for the lack of an oxygen increase in the inner retina during systemic hyperoxia. Instead, oxygen regulation is achieved by the remarkable ability of the choroid to maintain normal oxygen levels in the face of extreme systemic hyperoxia.[8] Thus, it appears that specific mechanisms are present in these mammals that serve to protect the retina from excessive oxygen levels. In the present study, we sought to determine whether there are similar mechanisms regulating oxygen delivery or oxygen consumption in the avascular region of the retina in the rabbit, a species routinely used in ophthalmic research, and in which increases in systemic oxygen levels result in severe toxic damage to the retina within a few days.[11–13]

Methods

Intraretinal Oxygen Profiles

The experimental techniques were similar to those reported in our earlier publications in the rabbit[14] and other species.[8,15] All procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Six adult rabbits of mixed breed were used. Oxygen-sensitive microelectrodes were used to measure the oxygen tension across the retina of anesthetized, mechanically ventilated rabbits under light-adapted conditions. Anesthesia was induced by an intramuscular injection of ketamine (50 mg/kg) and xylazine (3 mg/kg), followed by an intravenous infusion of ketamine (10 mg/kg) and xylazine (3 mg/kg) in 20 mL saline, infused at a rate sufficient to maintain anesthesia throughout the experiment. The animals were ventilated with air at 30 breaths per minute, with a tidal volume sufficient to produce blood gas levels within the normal range. Arterial blood pressure was monitored through a cannula in the femoral artery connected to a pressure transducer (CDX 3; Cobe Laboratories, Lakewood, CO) and recorded on a chart recorder. Before and after each profile measurement, the mean blood pressure was recorded to disk. We manufactured our own oxygen-sensing microelectrodes using techniques developed by Whalen et al.[16] The electrodes were calibrated in air-equilibrated saline before and after the experiment. The electrode entered the eye through a small hole just behind the limbus. The small size of the electrode tip (1 μm) coupled with high-acceleration piezoelectric translation of the electrode through the retina produced highly reproducible measurements of intraretinal oxygen distribution. Intraretinal oxygen profiles were measured in the avascular retina inferior to the disc, well away from the narrow horizontal band of retinal vessels present in the rabbit. The electrode tip was placed in front of the retina under microscope observation. The electrode was then stepped through the retina, under computer control, until a peak oxygen level within the choroid was reached. The measurement was then repeated during stepwise withdrawal of the electrode. Although very close agreement between the insertion and withdrawal profiles was routinely achieved, the withdrawal profiles were used for data analysis, because they tended to be less influenced by artifacts associated with mechanical stress on the electrode tip during penetration. Measurements were repeated after stepwise incremental (20%) increases in oxygen percentage in the ventilation gas, thus generating data for 20%, 40%, 60%, 80%, and 100% oxygen ventilation. At least 10 minutes were allowed for intraretinal oxygen levels to stabilize after each increase in systemic oxygenation, and each profile measurement

From the Centre for Ophthalmology and Visual Science, The University of Western Australia, Nedlands, Perth, Western Australia.

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Corresponding author: Stephen J. Cringle, Centre for Ophthalmology and Visual Science, The University of Western Australia, Nedlands, Perth, Western Australia 6009; cringle@cyllene.uwa.edu.au.

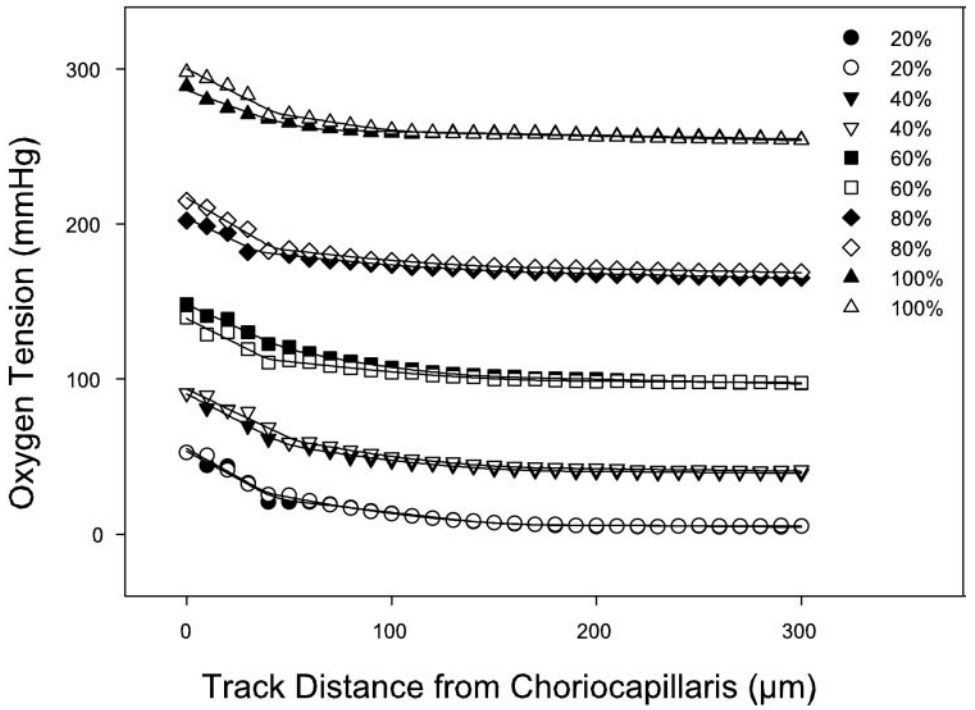

FIGURE 1. Intraretinal oxygen distribution in the avascular area of a rabbit before and after stepwise increases in the percentage of oxygen in the ventilation mixture (20%, 40%, 60%, 80%, and 100% oxygen). The data show oxygen tension as a function of electrode track distance from the choriocapillaris. *Solid lines*: best fit of the data to the mathematical model of oxygen consumption. Two measurements were obtained for each ventilation condition. Each increase in inspired oxygen percentage resulted in an increase in choriocapillaris oxygen level and a similar increase in all retinal layers.

took approximately 3 minutes. Typically, two intraretinal profiles were measured under each condition, and all profiles were used in the subsequent oxygen-consumption analysis.

Mathematical Models

For the analysis of the oxygen profiles, a modified form of the model of Haugh et al.[17] was used. The model was expanded to include five layers, which allowed us to separate out the oxygen consumption rates of the outer retina (Q_{or}), and the inner retina (Q_{ir}), along with the oxygen gradients in diffusion zones at the boundaries with the choroid and vitreous. Total retinal oxygen consumption (Q_{total}) is the sum of Q_{or} and Q_{ir}. This model has been applied to the avascular area of the rabbit retina in determining baseline oxygen consumption values of the inner and outer retina.[14]

Statistics

All statistical testing was performed on computer (SigmaStat; SPSS Scientific; Chicago, IL). All data are expressed as the mean ± SE, and all error bars on graphs are also standard errors. The paired *t*-test was used to test the significance of changes in oxygen consumption at different oxygen ventilation levels. One-way repeated-measures ANOVA was used to test the significance of changes in oxygen tension in the choroid and the innermost retina after an incremental increase in the percentage of inspired oxygen. The significance level was set at $P < 0.05$.

RESULTS

A representative set of intraretinal oxygen profiles for each ventilation condition in a single animal is shown in Figure 1. The measurement points are shown along with the best fit of the consumption model to the data. With each increment in inspired oxygen level, there was an increase in choroidal oxygen level and a similar increase across the entire retina. There were similar responses to systemic hyperoxia in all animals tested. The average data for all animals ($n = 6$) are shown in Figure 2. The increase in choroidal PO_2 was significant for each increment in oxygen ventilation level, as was the increase in all retinal layers and in the vitreous in front of the retina. The mean systemic arterial blood pressure for each animal was determined by averaging all the blood pressure measurements before and after each profile measured. The average mean systemic arterial blood pressure across all the animals was 79.0 ± 6.3 mm Hg. There was no significant effect of oxygen ventilation on mean blood pressure. The corresponding blood pressure levels (mm Hg) were 83.0 ± 5.4 at 20% oxygen, 79.2 ± 4.3 at 40%, 80.8 ± 5.7 at 60%, 81.3 ± 3.1 at 80%, and 77.2 ± 3.8 at 100% oxygen.

Figure 3 shows the calculated oxygen consumption values for the outer and inner retina of each animal at each ventilation condition, along with the total retinal oxygen consumption in each case. The best linear fit between the oxygen consumption data and the inspired oxygen level is shown for each animal. There was no statistically significant change in inner retinal oxygen consumption with increasing oxygen ventilation levels. Outer retinal oxygen consumption ($P = 0.007$) and total retinal oxygen consumption ($P = 0.01$) were significantly reduced only at the 100% oxygen ventilation level when compared with normal (20%) ventilation conditions.

No correction was made for the nonperpendicular nature of the electrode track through the retina. Applying such a correction would increase the oxygen consumption slightly, but the effect would be the same for all profiles measured in the same location.

An example of the magnitude and timing of the PO_2 increase in the vitreous close to the retinal surface is shown in Figure 4 for an animal switched directly from 20% to 100% oxygen ventilation before any electrode penetration of the retina. The increase in preretinal oxygen tension was both large (250 mm Hg) and rapid (33 seconds to half amplitude).

DISCUSSION

An insufficient supply of oxygen to the retina is thought to be an important pathogenic factor in a variety of retinal diseases with an ischemic component. Oversupply of oxygen can also result in retinal disease, such as retinopathy of prematurity. There is also evidence that oxygen toxicity plays a role in

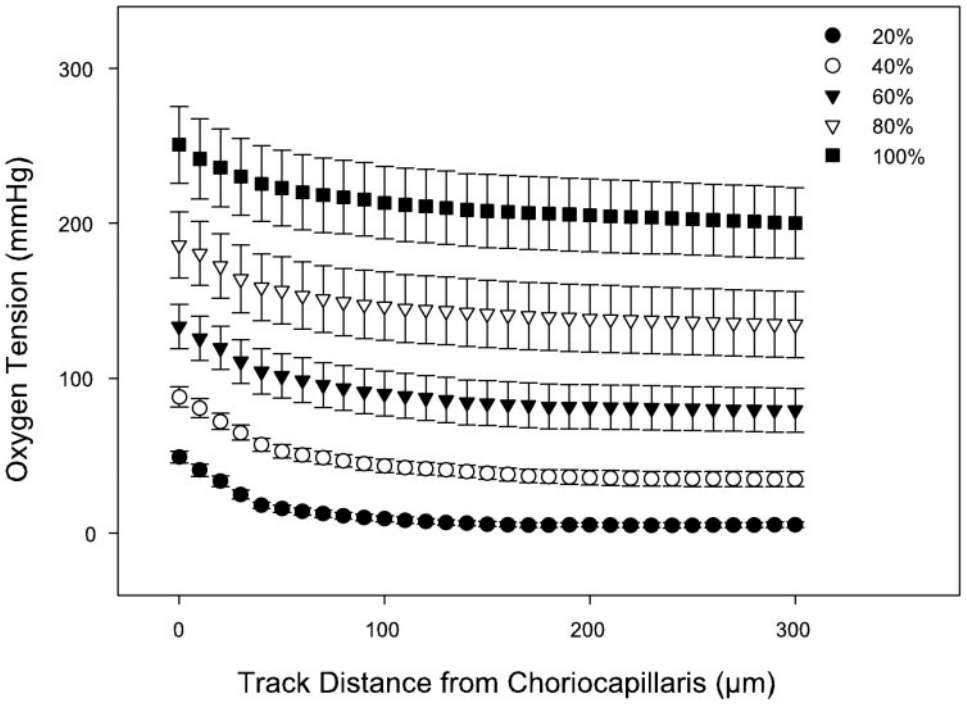

FIGURE 2. Average data from all animals studied ($n = 6$). The mean oxygen level as a function of electrode track distance from the choriocapillaris is shown for each ventilation mixture (20%, 40%, 60%, 80%, and 100% oxygen). Each increase in inspired oxygen percentage results in an increase in choriocapillaris oxygen level and a similar increase in all retinal layers. Error bars, SEM.

degenerative retinal diseases, such as retinitis pigmentosa (RP),[18] and it has been suggested that manipulation of the retinal oxygen environment may be a therapeutic tool in the management of retinal diseases, such as RP,[19] retinal detachment, and occlusive diseases of the retinal circulation. Even in healthy eyes, there is a very delicate balance between oxygen supply and consumption. Disruption of this balance may cause too little or too much oxygen to be present in specific retinal layers. Understanding the oxygen requirements of different components of the retina is vital if therapeutic strategies to restore an appropriate oxygen environment are to be developed.

In microelectrode-based studies in monkeys,[1] pigs,[3] cats,[4–7] guinea pigs,[8] and rats[9] the increase in inner retinal oxygen tension during systemic hyperoxia was much less than one would expect, given the increase in systemic arterial oxygen levels. These finding are summarized in Figure 5 where the magnitude of the PO_2 increase at the retinal surface when switching from 20% to 100% oxygen ventilation is shown for a range of species. Where intraretinal measurements were not available, the size of the preretinal PO_2 response was used as the estimate of the change at the retinal surface.

Pournaras et al.,[3] based on their findings in the pig, speculated that increased oxygen consumption in the outer retina could be responsible for the muted inner retinal oxygen change during systemic hyperoxia. However, Linsenmeier and Yancey[4] found that outer retinal oxygen consumption in the cat did not increased during systemic hyperoxia. More recent work from our laboratory has uncovered specific mechanisms regulating the intraretinal oxygen environment in the rat.[9,10] It was shown that excessive increases in inner retinal oxygen tension during systemic hyperoxia are prevented by a combination of reduced oxygen input from the deep capillary layer and an increase in inner retinal oxygen consumption.[10] In the avascular retina of the guinea pig, it has been shown that the choroid is the major regulator of oxygen level throughout the retina and that intraretinal oxygen levels are largely unaffected by systemic hyperoxia.[8] Disabling this mechanism by inducing systemic hypercapnia along with hyperoxia results in increased levels of oxygen tension throughout the retina.[8] Thus, in many mammals studied to date, there appears to be mechanisms aimed at protecting some regions of the retina from exposure to excessive oxygen levels. The existence of such mechanisms is indicative that they are important for maintaining healthy retinal function. We sought to extend this work by examining the behavior of the retina of the rabbit, an animal with a predominantly avascular retina widely studied in other aspects of ophthalmic research. Our findings indicate that the rabbit does not possess any significant oxygen-regulating ability in the face of systemic hyperoxia. High oxygen levels were found throughout the retina and choroid during systemic hyperoxia. This is in agreement with the vitreal measurements of oxygen tension in the rabbit during 100% oxygen ventilation[20,21] and with the pilot data that we described in an earlier report.[22] The time scale of the vitreal response in our study is much faster than that demonstrated by Wilson et al.,[21] presumably reflecting the closeness of our measurement site to the retinal surface and their requirement for oxygen levels to equilibrate in the bubble of perfluorocarbon within the vitreous. It is worth noting that in the rabbit the increase in preretinal oxygen tension during hyperoxic ventilation is effectively the same as seen in all layers of the retina. This allows the intraretinal oxygen changes to be predicted from the preretinal oxygen response, the measurement of which is amenable to recently developed noninvasive techniques.[23]

A limitation of the present study is that intraocular pressure (IOP) may have been less than normal. IOP was not monitored, but the absence of a pressure-tight seal at the entry point for the microelectrode could lead to some degree of hypotony. However, we argue that this would be unlikely to influence the results of our study significantly. Identical techniques were used in our earlier studies in the guinea pig, in which tight regulation of choroidal PO_2 was observed during systemic hyperoxia,[8] and so impairment of choroidal regulation due to the measurement technique seems unlikely. Furthermore, our preretinal oxygen response data are in very close agreement with noninvasive studies in the rabbit in which steps were taken to ensure normal IOP.[21] We did not make measurements of choroidal blood flow, but others have shown that systemic hyperoxia does not result in altered choroidal blood flow in

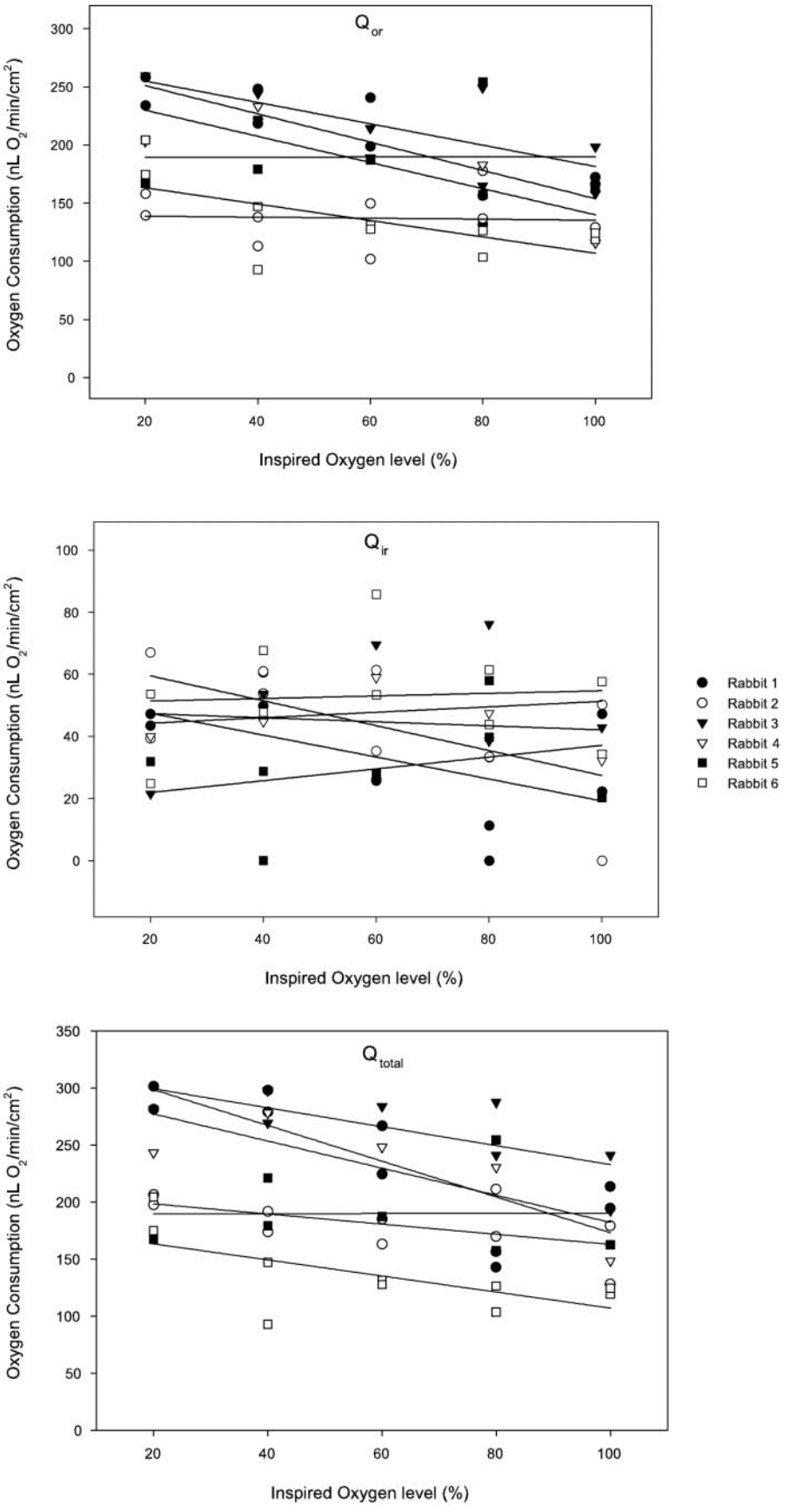

FIGURE 3. Calculated oxygen consumption values for the outer (Q_{or}), inner (Q_{ir}), and whole (Q_{total}) retina for each of the six animals under each ventilation condition. The best linear fit between oxygen consumption and the level of inspired oxygen is shown for each animal. The only change reaching statistical significance was that Q_{or} and Q_{total} were significantly reduced during 100% oxygen ventilation.

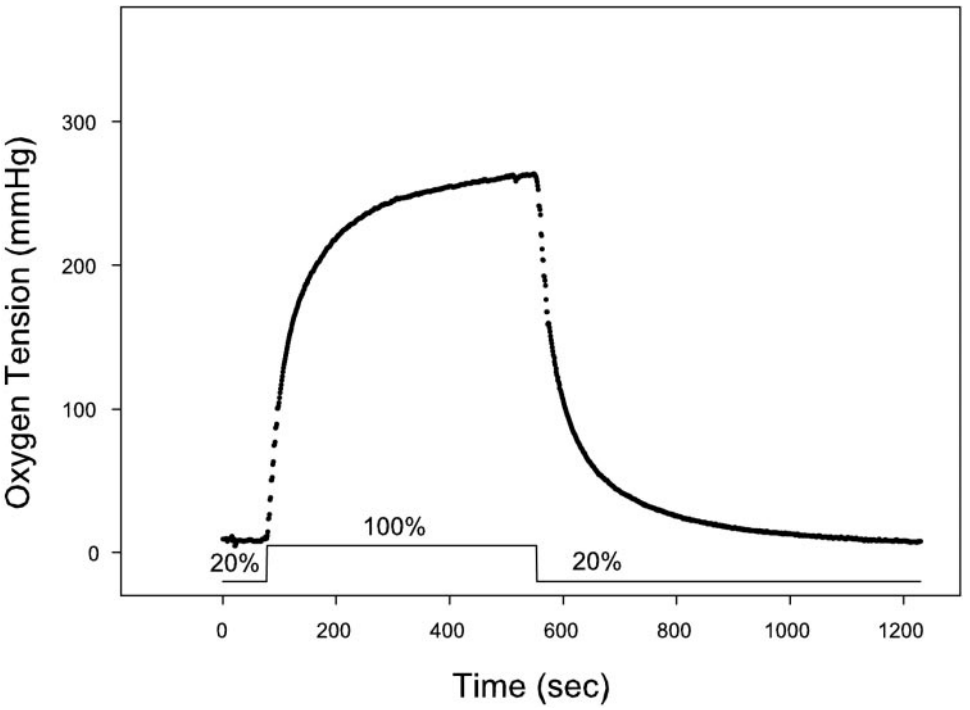

FIGURE 4. An example of the timing and magnitude of the preretinal increase in oxygen tension in front of the avascular area of the rabbit retina after a transition from 20% to 100% oxygen ventilation. Oxygen tension is shown as a function of time. The *lower trace* indicates the timing and duration of the 100% oxygen exposure. In this example, the time to reach half the maximum amplitude of the response was 33 seconds, and the peak increase in PO_2 was 250 mm Hg.

humans or cats.[24,25] It seems likely that the rabbit choroid is similarly unresponsive to systemic hyperoxia.

A question that arises from the results of the present study is whether the rabbit retina is more prone to oxygen toxicity during systemic hyperoxia than other species in which inner retinal oxygen levels are more tightly regulated. There is histologic and electrophysiological evidence to suggest that the rabbit retina is particularly vulnerable to oxygen exposure.[11,13] Noell[11] in 1955 reported that more than 70% of the visual cells were degenerated when the rabbit was in an ambient pressure oxygen environment for only 48 hours. No such effects are seen in longer-term oxygen exposure in cats or mice. The rabbit therefore appears to provide a valuable opportunity to study oxygen-induced retinal damage. Toxic effects are rapid and extensive, the intraretinal oxygen environment follows a relatively simple relationship to the environmental oxygen exposure, and all retinal layers are subjected to a similar increase in oxygen level. In the rabbit, there appears to be no

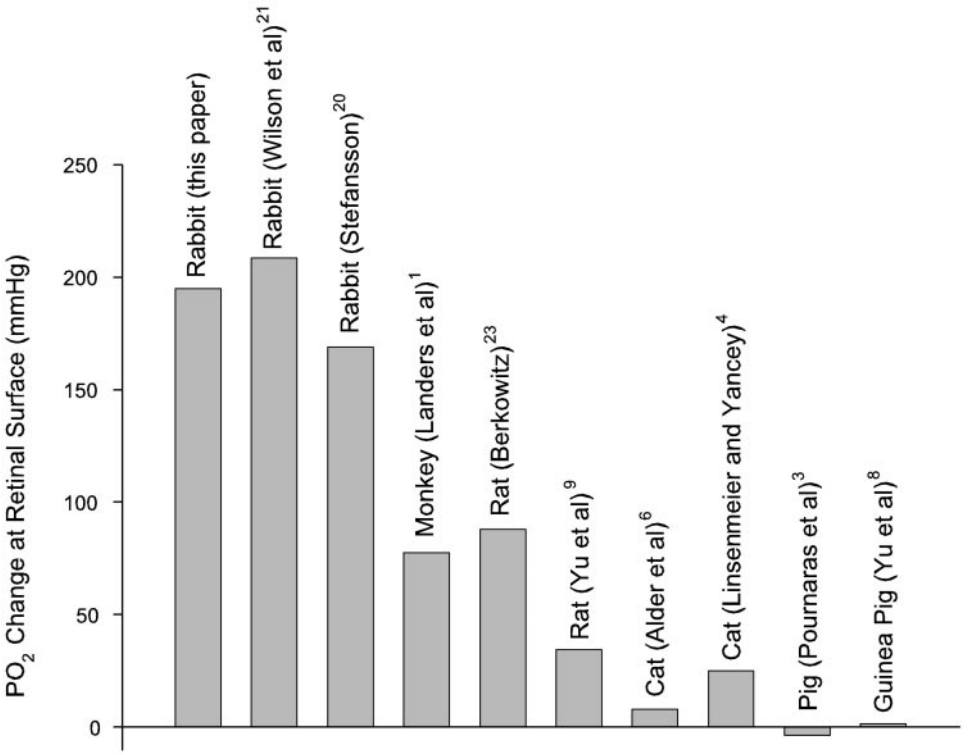

FIGURE 5. Summary of measured increases in PO_2 in the innermost retina when switching from air to 100% oxygen ventilation in a range of species (rabbit, monkey, cat, pig, and guinea pig). Preretinal measurements are used as an estimate of innermost retinal PO_2 where intraretinal measurements were not available.

regulatory mechanisms to ameliorate the extent of oxygen exposure in the retina during systemic hyperoxia.

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