Optic nerve oxygen tension: effects of intraocular pressure and dorzolamide

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Abstract

Aim—To investigate the influence of acute changes in intraocular pressure on the optic nerve oxygen tension in the vicinity of the optic nerve head under control conditions and after intravenous administration of 500 mg of the carbonic anhydrase inhibitor dorzolamide.

Methods—Domestic pigs were used as experimental animals. Oxygen tension was measured by means of a polarographic electrode in the vitreous 0.5 mm anterior to the optic disc. This entity is called the optic nerve oxygen tension. Intraocular pressure was controlled by a hypodermic needle inserted into the anterior chamber and connected to a saline reservoir.

Results—When the intraocular pressure was clamped at 20 cm H$_2$O optic nerve oxygen tension was 20 (5) mm Hg (n=8). Intravenous administration of dorzolamide caused an increase in optic nerve oxygen tension of 43 (8)% (n=6). Both before and after administration of dorzolamide optic nerve oxygen tension was unaffected by changes in intraocular pressure, as long as this pressure remained below 60 cm H$_2$O. At intraocular pressures of 60 cm H$_2$O and below, dorzolamide significantly increased optic nerve oxygen tension.

Conclusion—Intravenous administration of 500 mg dorzolamide increases the oxygen tension at the optic nerve head during acute increases in intraocular pressure.

Glaucoma is a group of diseases that has been classically characterised by high intraocular pressure (IOP), damage to the optic nerve head, and corresponding visual field defects. Currently, elevated IOP is considered one of many risk factors for glaucomatous optic nerve damage. Increased IOP may directly injure the optic nerve fibres, or it may compromise vascular supply and thereby indirectly damage the optic nerve.  

There is overwhelming evidence that, in healthy experimental animals, blood flow in the optic nerve head is autoregulated and is therefore largely unaffected by acute increases in IOP up to 50 mm Hg or more.  

Materials and methods

Anaesthesia and animal preparation

Danish domestic pigs (Danish Landrace), 25–33 kg in weight, were used as experimental animals. Their treatment was supervised by a veterinarian nurse and followed the ARVO resolution for the use of animals in ophthalmic and vision research. Permission for the use of pigs in this study was granted by the Danish board for surveillance of the use of experiments.
Intraocular pressure was controlled by adjusting the height of a saline reservoir connected to a 27 gauge hypodermic needle inserted into the anterior chamber.

**OXYGEN MEASUREMENTS**

Optic nerve oxygen tension was measured with a polarographic oxygen electrode that consists of a 100 µm platinum-iridium electrode inside a 20 gauge needle (Model 761, Diamond Electrotech Inc, Ann Arbor, MI, USA). The polarographic electrode was advanced through the vitreous cavity placed approximately 2.0 mm behind the limbus. Once in the vitreous cavity, the electrode was mounted in a micromanipulator and visualised by indirect ophthalmoscopy. The tip of the polarographic electrode was placed 0.5 mm above the optic nerve head in an area devoid of major vessels. A silver-silver chloride (Ag/AgCl) reference electrode was placed subcutaneously in the lower lid.

The polarographic oxygen electrode was calibrated before each experiment in a calibrating cell containing 155 mM saline bubbled with 100% N₂, 5%O₂,95%N₂, and 20%O₂/80%N₂ at 37°C and used as described by Stefánsson et al. The oxygen tension recordings were adjusted for barometric pressure and expressed as mm Hg.

The experiments were all run with room lights on (white fluorescent light tubes). The ambient light intensity at the level of the pig’s eye was found to be approximately 500 lux.

**EXPERIMENTAL PROTOCOL**

Seven out of eight experiments were begun with the animal breathing 100% oxygen. Once a stable optic nerve oxygen tension was obtained, the breathing mixture was changed to ambient air containing 21% oxygen. In one experiment the pig breathed air throughout the experiment. While the pig was ventilated with ambient air, and after stabilisation of optic nerve oxygen tension, the IOP was clamped at 20 cm H₂O and subsequently changed in steps to 60, 80, 100, and 120 cm H₂O (or 15, 0, 15, 29, 44, 59, 74, and 88 mm Hg) was tested. The IOP was kept constant until optic nerve oxygen tension had stabilised, in most cases for 3–5 minutes. Seven out of eight experiments the effect of dorzolamide was tested. After the initial sequence of changes in IOP, the IOP was changed back to 20 cm H₂O and 500 mg dorzolamide (16.7 ml of a 3% solution) was administered in the femoral vein. After stabilisation of optic nerve oxygen tension, IOP was again changed as already described and the resulting changes in optic nerve oxygen tension were recorded.

**DATA ANALYSIS**

Individual values of IOP were tested between one and seven times in each pig. There was a considerable variation in the values of optic nerve oxygen tension between pigs. In order to...
The number of pigs (n), and the p value for the paired t test are indicated.

<table>
<thead>
<tr>
<th></th>
<th>Before dorzolamide</th>
<th>After dorzolamide</th>
<th>n</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial blood pH (units)</td>
<td>7.64 (0.05)</td>
<td>7.49 (0.04)</td>
<td>6</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Arterial blood PCO₂ (kP)</td>
<td>3.4 (0.4)</td>
<td>5.1 (0.5)</td>
<td>6</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Arterial blood PO₂ (kP)</td>
<td>13 (1)</td>
<td>13 (1)</td>
<td>6</td>
<td>0.3</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>81 (7)</td>
<td>84 (6)</td>
<td>6</td>
<td>0.09</td>
</tr>
</tbody>
</table>

The number of pigs (n), and the p value for the paired t test are indicated.

Results

In seven pigs the animal was initially ventilated with pure oxygen. When the inspired gas was changed from pure oxygen to air (21% oxygen), the optic nerve oxygen tension decreased from a mean value of 41 (SE 7) mm Hg to 21 (4) mm Hg. This is similar to our previously reported finding.

Figure 1 shows a typical experiment where a sequence of increasing IOPs was investigated. It is seen that optic nerve oxygen tension remained relatively constant for IOP up to 40 cm H2O. Only after the IOP was increased to 80 cm H2O did optic nerve oxygen tension drop precipitously. Above 120 cm H2O further increases in IOP did not influence optic nerve oxygen tension. The mean arterial blood pressure was around 70 mm Hg during this experiment. Note the large overshoot in optic nerve oxygen tension after the IOP returned from 140 cm H2O to 20 cm H2O. After challenge with high values of IOP we kept the IOP at 20 cm H2O until optic nerve oxygen tension had stabilised (15–60 minutes). The optic nerve oxygen tension observed after runoff of these overshoots was similar to that measured in the same pig at the beginning of the experiments, before the IOP had been changed. The difference was 5 (7) mm Hg (n=8) which is not statistically significantly different from zero (paired t test, p>0.1).

In six pigs the effects of dorzolamide were studied. The results of intravenous administration of 500 mg dorzolamide on arterial pH, arterial oxygen and carbon dioxide tensions, and mean arterial blood pressure are shown in Table 1. Dorzolamide caused an increase in arterial carbon dioxide tension and a corresponding decrease in arterial pH, but no changes in mean arterial blood pressure or in arterial oxygen tension. These changes are similar to our previously reported findings.

Administration of dorzolamide was performed while the IOP was clamped at 20 cm H2O. After administration of dorzolamide the optic nerve oxygen tension increased by a mean of 43 (SE 8)% (n=6). This is similar to our previously reported finding in experiments where the IOP was not clamped.

When we compared the six experiments where the animals were relaxed and anaesthetised with mebumal and fentanyl and the two experiments where the animals were not relaxed and were anaesthetised with alphachloralose we found no differences in the effects of dorzolamide and the relation between IOP and optic nerve oxygen tension. Thus, the described findings seem to be independent of the anaesthesia used.

Figure 2 summarises the results of the experiments similar to that shown in Figure 1, performed before and after administration of dorzolamide. The optic nerve oxygen tension was largely unaffected by changes in IOP as long as this was below 60 cm H2O (Fig 2). This was the case both before and after administration of dorzolamide. Figure 2 includes all data in the study, including our first two pigs in whom dorzolamide was not administered. For unknown reasons these two pigs had smaller optic nerve oxygen tensions than the subsequent six pigs, all of which received the drug. In the two pigs not given dorzolamide the mean optic nerve oxygen tension measured at an IOP of 20 cm H2O was 14 (10) mm Hg. In the remaining six pigs which did receive the drug the optic nerve oxygen tension was 22 (6) mm Hg. Figure 2 shows the influence of the IOP on optic nerve oxygen tension under control conditions and after administration of dorzolamide. However, at any given value of IOP, Figure 2 overestimates the effects of the drug on optic nerve oxygen tension.

To obtain a true assessment of the effects of dorzolamide on the optic nerve oxygen tension we compared the optic nerve oxygen tensions measured before and after administration of the drug in the same pig. Figure 3 shows these paired differences in optic nerve oxygen tension for each of the investigated values of IOP. Dorzolamide significantly increased optic nerve oxygen tension for IOP values of 60 cm H2O and below.

Discussion

We have previously shown that it is possible to make reliable measurements of the oxygen tension in the normal pig vitreous using the relatively large polarographic oxygen electrode.
used in the present study. The vitreous acts as a diffusion medium, with very low endogenous oxygen consumption. The oxygen gradients within the vitreous are small (about 2 mm Hg per mm distance). The oxygen tension in vitreous 0.5–1.0 mm in front of the optic nerve is therefore relatively insensitive to the exact distance of the sensor to the optic nerve head, and we interpret the measured oxygen tension as reflecting the average oxygen tension in the superficial part of the optic nerve head, and denote it optic nerve oxygen tension.

Earlier measurements with microelectrodes have shown that the oxygen tension in front of the optic nerve head is unevenly distributed. In a study in minipigs the oxygen tension in the vitreous was 33 mm Hg during normoxia and increased to 81 mm Hg during hyperoxia (100% oxygen). In vitreous overlying the optic nerve head, but not adjacent to arterioles, the oxygen tension was 17 mm Hg and remained unchanged during hyperoxia. Our measurements probably represent a spatial average of optic nerve head oxygen tensions, although some contribution from the adjacent retina cannot be excluded. We carefully placed the electrode over an area without major vessels. Accordingly, we found oxygen tensions between previously reported values for vitreal oxygen tension measured adjacent to optic nerve head arterioles, and oxygen tension measured away from such arterioles. Both during normoxia and hyperoxia, our values were closest to previously reported oxygen tensions over optic nerve head tissue away from major vessels.

In our experiments the oxygen tension in arterial blood remained unchanged after intravenous administration of dorzolamide (Table 1). It is therefore not likely that the changes in optic nerve oxygen tension observed after administration of this drug were solely caused by altered diffusion of oxygen out of arterioles.

The measured changes in optic nerve oxygen tension could be caused by changes in oxygen delivery or changes in oxygen consumption (metabolism). We assume that changes in oxygen delivery were responsible for the major part of the observed changes in optic nerve oxygen tension. We have previously found that the optic nerve oxygen tension increases as expected when the optic nerve head blood vessels are diluted by the well known CNS vasodilators acetazolamide and carbon dioxide. We have also reported that the changes in optic nerve oxygen tension closely reflect changes in blood flow in the optic nerve head, as measured by a laser Doppler flow probe.

The present experiments show that the optic nerve oxygen tension in the pig is autoregulated. This autoregulation is most obvious when the vessels have been dilated by the prior administration of dorzolamide. Only at IOP above 60 cm H2O did we observe a precipitous drop in the optic nerve oxygen tension. An IOP of 60 cm H2O corresponds to an intraocular perfusion pressure of approximately 35 mm Hg if this entity is defined as the mean arterial blood pressure minus the IOP. With the IOP increased to 120 cm H2O the intraocular perfusion pressure was around 7 mm Hg. With this IOP we found optic nerve oxygen tensions not significantly different from zero under control conditions. After administration of dorzolamide there might have been some pulsatile flow during the systole, since positive optic nerve oxygen tensions were measured (Fig 2). The present findings are in agreement with previous studies of autoregulation of blood flow in the optic nerve head.

We have previously shown that intravenous administration of the carbonic anhydrase inhibitors acetazolamide and the more potent dorzolamide increase the optic nerve oxygen tension in a dose dependent manner. The present experiments indicate that carbonic anhydrase inhibition does not inhibit autoregulation of the optic nerve oxygen tension. Instead, after carbonic anhydrase inhibition this autoregulation is preserved, but the level of optic nerve oxygen tension is shifted upwards (Fig 2).

At all values of IOP tested the optic nerve oxygen tension was higher after administration of dorzolamide than in controls (Fig 3). The present experiments confirm earlier preliminary findings that the effects of dorzolamide on the optic nerve oxygen tension is a direct effect of the drug on the optic nerve head circulation, rather than an indirect effect due to a lowering of the IOP.

Dorzolamide was administered only after a series of raised IOPs had been tested in the absence of the drug. The advantage of this approach is that each pig can serve as its own control in assessing the effects of the drug. However, it may be argued that local vasodilators are released during the period of high IOP and that their effects are present during the remaining course of the experiment. This would cause an overestimation of the vasodilating effect of dorzolamide. We cannot rule out this possibility. However, after a series of raised IOPs had been tested the optic nerve oxygen tension was dramatically increased by the administration of dorzolamide. We have previously shown that intravenous administration of acetazolamide or carbonic anhydrase inhibition increases optic nerve oxygen tension in a dose dependent manner.

Once the drug effects were assessed, dorzolamide was administered only after a series of raised IOPs had been tested in the absence of the drug. The advantage of this approach is that each pig can serve as its own control in assessing the effects of the drug. However, it may be argued that local vasodilators are released during the period of high IOP and that their effects are present during the remaining course of the experiment. This would cause an overestimation of the vasodilating effect of dorzolamide. We cannot rule out this possibility. However, after a series of raised IOPs had been tested the optic nerve oxygen tension was dramatically increased by the administration of dorzolamide. We have previously shown that intravenous administration of acetazolamide or carbonic anhydrase inhibition increases optic nerve oxygen tension in a dose dependent manner.

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tension stabilised at values that were not statistically significantly different from those found at the beginning of the experiments before the IOP was altered.

The vasculature of the optic nerve head in the pig has many similarities to that in humans but it is more clearly trilaminar with a more well defined arterial circle of Zinn-Haller. If the findings of the present investigation can be generalised to the human optic nerve head circulation, intravenous dorzolamide, and perhaps other carbonic anhydrase inhibitors too, may be useful in preserving oxygenation of the optic nerve head during acute episodes of increased IOP in otherwise healthy young eyes. However, it must be remembered that these experiments were carried out in healthy young pigs and have only a theoretical bearing on the situation in patients with glaucoma in whom the optic nerve head circulation and its regulation may have been damaged by the disease.

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