Optic nerve oxygen tension: the effects of timolol and dorzolamide

J F Kiigaard, D B Pedersen, T Eysteinsson, M la Cour, K Bang, P K Jensen, E Stefánsson

MATERIALS AND METHODS
Domestic pigs (Danish Landrace) (n = 15), 28–30 kg in weight and brought up in a specific pathogen free environment, 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 Dyreforsøgsstilsynet (Danish Animal Experiments Inspectorate).

Dorzolamide HCl and timolol eye drops (Timacar) was obtained from Merck, Sharp & Dohme (Glostrup, Denmark). Dorzolamide was dissolved as a 3% solution in 100 mM citrate buffer, pH 5.6.

Anaesthesia and animal preparation
Anaesthesia was induced by an intramuscular injection of 15 mg midazolam (Dumex-Alpharma) followed by 3 ml of a mixture of tiletamin 25 mg/ml and zolazepam 25 mg/ml (Zoletil 50 vet, Boehringer Ingelheim), xylazin 13 mg/ml (Narcoxyl vet, Veterinaria AG), ketalar 15 mg/ml (Ketaminol, Veterinaria AG), and methadone 2.5/ml (Methadon DAK, Nycomed). After induction, the pigs were intubated, and artificially ventilated with air. Catheters were placed in the left femoral artery, left femoral vein, and in the left cranial superficial epigastrical vein. During the experiment, anaesthesia was maintained by infusion of pancuronium bromide 8 mg/hour (Pavulon, Organon) and fentanyl 400 μg/hour (Fentanyl, Dumex-Alpharma) in one vein and pentobarbital 300 mg/hour (membros, Den Kongelige Veterinær-og Landbohøjskoles Apotek, Denmark) in the other vein.

Heart rate (HR) and mean arterial blood pressure (MAP) was monitored throughout the experiments.

The pig was placed in a sling and the head additionally secured stereotactically. A speculum was placed between the eyelids of the left eye. The pupil of this eye was dilated with 1% atropine eye drops (Atropin SAD, Sygehusapotekerne i DK), 0.4% oxybuprocaine (Oxybuprokan SAD, Sygehusapotekerne i DK), 1% cyclopentolate (Cyclogyl, Alcon), and 2.5% methoxaodrine (methoxaodrine SAD, Sygehusapotekerne i DK). Two 4–0 silk traction sutures were placed in the sclera to immobilise the eye. A sclerotomy was made 2.0 mm behind the limbus in the superior nasal quadrant and a plastic cannula (16 gauge) was placed in the sclerotomy. To avoid contamination of the electrode with blood products, it was advanced through the cannula.

Oxygen tension measurements
The oxygen tension over the optic nerve was measured with a polarographic oxygen electrode with an internal Ag/AgCl reference electrode, embedded in a 20 gauge needle (model 768–20R, Diamond General Development Corporation, Ann Arbor, MI, USA). The electrode was calibrated and the optic nerve oxygen tension measurement were performed as previously described.12
Experimental protocol
Frequent arterial blood samples were drawn from the catheter in the femoral artery, and analysed for oxygen and carbon dioxide tensions and pH, using an ABL 605 blood gas analyser (Radiometer, Copenhagen, Denmark). The respirator was adjusted in stroke volume and frequency to ensure normal blood PO2 (apH) (10–14 kPa), PCO2 (aPCO2) (5.5–7.5 kPa) and pH values (apH) (7.38–7.42) according to the blood samples.

To test the oxygen electrode, the pig was initially given 100% oxygen in the inspiratory air for 10 minutes. After having obtained a stable oxygen tension recording, five pigs were given intravenous injections of 100 mg timolol initially and 500 mg dorzolamide 35 minutes later. Five pigs were given intravenous injections of 500 mg dorzolamide and 100 mg timolol 35 minutes afterwards, and five pigs were given a combination of 500 mg dorzolamide and 100 mg timolol intravenously simultaneously. Arterial blood samples were drawn at following time points according to the different drug injections: −1 minute, 1 minute, 10 minutes, and 30 minutes.

The mean total duration of the experiments was 4 hours and 47 (SD 88) minutes (n = 15). The electrode recordings lasted 3 hours and 22 (SD 88) minutes (n = 15). The electrode recordings were drawn at following time points according to the different drug injections: −1 minute, 1 minute, 10 minutes, and 30 minutes.

The mean standard deviation of ONPO2, aPO2, aPCO2, aPCO2, MAP, and HR were calculated (table 1).

Data processing
ONPO2, MAP, and HR values (averaged over 15 seconds in the Axoscope recording) for baseline and time points after injection of the study drugs were used for all the calculations.

The mean and standard deviation of ONPO2, aPO2, aPCO2, aPCO2, MAP, and HR were calculated (table 1).

Differences in the parameters before and after drug injections in each pig were tested for significance using Student’s paired two tailed t test. Difference between groups was tested for significance using Student’s unpaired two tailed t test. A significance level of 0.05 was used.

All results (table 1) were presented as mean (SD) (n = number of experiments).

Graphic presentations of the tracings were made in SigmaPlot 2001 for Windows (Version 6.00 SPSS Inc, Chicago, IL, USA).

RESULTS
Timolol injection had no effect on ONPO2 (fig 1A). Baseline ONPO2 was 4.33 (0.25) kPa (n = 5, table 1). This decreased insignificantly by 0.11 (0.16) kPa after timolol injection (n = 5, p = 0.194, figs 1A and 2). Injection of dorzolamide increased ONPO2 significantly by 0.72 (0.39) kPa (n = 4, p = 0.035, figs 1B and 2). One experiment with injection of dorzolamide was excluded because of movement of the pig head; however, the measurements during the following injection of timolol could be performed satisfactorily and was included. When timolol was given to the dorzolamide treated pigs, ONPO2 increased insignificantly by 0.19 (0.30) kPa (n = 5, p = 0.303, fig 1B). Dorzolamide and timolol given simultaneously elevated ONPO2 significantly by 0.83 (0.18) kPa (n = 5, p<0.001, figs 1C and 2) and a similar increase was seen when dorzolamide was given to the timolol treated animal (0.86 (0.34) kPa (n = 5, p<0.001, figs 1A and 2)). There was no significant difference between the effects of injection of 500 mg dorzolamide when it was given in control animals, in timolol treated animals, or simultaneously with timolol (fig 2).

Table 1

<table>
<thead>
<tr>
<th>100 mg timolol iv (alone)</th>
<th>500 mg dorzolamide iv (alone)</th>
<th>500 mg dorzolamide iv (after timolol)</th>
<th>100 mg timolol iv (after dorzolamide)</th>
<th>500 mg dorzolamide+100 mg timolol iv</th>
</tr>
</thead>
<tbody>
<tr>
<td>ONPO2 (kPa)</td>
<td>4.33 (0.25)</td>
<td>4.23 (0.19)</td>
<td>2.96 (0.62)</td>
<td>4.00 (0.90)</td>
</tr>
<tr>
<td>ONPO2 (30 min) (kPa)</td>
<td>4.22 (0.19)</td>
<td>5.09 (0.48)†</td>
<td>3.69 (0.88)†</td>
<td>4.19 (1.11)†</td>
</tr>
<tr>
<td>aPH (baseline)</td>
<td>7.40 (0.01)</td>
<td>7.41 (0.01)</td>
<td>7.41 (0.01)</td>
<td>7.33 (0.02)</td>
</tr>
<tr>
<td>aPH (30 min)</td>
<td>7.41 (0.01)</td>
<td>7.33 (0.02)†</td>
<td>7.34 (0.01)†</td>
<td>7.32 (0.01)†</td>
</tr>
<tr>
<td>aPCO2 (baseline) (kPa)</td>
<td>7.2 (0.6)</td>
<td>7.0 (0.6)</td>
<td>7.2 (0.4)</td>
<td>8.4 (0.5)</td>
</tr>
<tr>
<td>aPCO2 (30 min) (kPa)</td>
<td>7.0 (0.6)</td>
<td>8.4 (0.5)†</td>
<td>8.2 (0.4)†</td>
<td>8.6 (0.4)†</td>
</tr>
<tr>
<td>aPCO2 (baseline) (kPa)</td>
<td>10.6 (1.0)</td>
<td>10.1 (0.8)</td>
<td>11.9 (1.5)</td>
<td>10.9 (2.1)</td>
</tr>
<tr>
<td>aPCO2 (30 min) (kPa)</td>
<td>10.1 (0.8)</td>
<td>10.2 (0.8)</td>
<td>12.3 (1.4)</td>
<td>10.9 (2.2)</td>
</tr>
<tr>
<td>MAP (baseline) (mm Hg)</td>
<td>114 (16)</td>
<td>108 (11)</td>
<td>99 (20)</td>
<td>84 (27)</td>
</tr>
<tr>
<td>MAP (30 min) (mm Hg)</td>
<td>110 (12)</td>
<td>99 (16)</td>
<td>89 (23)†</td>
<td>77 (26)†</td>
</tr>
<tr>
<td>HR (baseline)</td>
<td>86 (7)</td>
<td>89 (5)</td>
<td>99 (20)</td>
<td>86 (11)</td>
</tr>
<tr>
<td>HR (30 min)</td>
<td>85 (8)†</td>
<td>89 (23)</td>
<td>89 (11)</td>
<td>87 (21)</td>
</tr>
</tbody>
</table>

* A statistically significant difference (p<0.05) between baseline and 30 minutes values, tested with Student’s t test.
MAP and HR did not change after the injections of timolol, but decreased slightly in some of the groups when dorzolamide was injected (table 1). Injection of timolol did not affect aPH and aPCO₂. However, an injection of dorzolamide created a metabolic acidosis, decreasing aPH and increasing aPCO₂ (table 1). Arterial PO₂ did not change in any of the experiments.

**DISCUSSION**

There were two new findings in this study. Firstly, timolol injected intravenously had no significant effect on ONPO₂. Secondly, the effect of dorzolamide on ONPO₂ was not affected by timolol injection given neither before, in combination, nor after dorzolamide.

Injection of timolol did not affect aPH and aPCO₂. However, an injection of dorzolamide created a metabolic acidosis, decreasing aPH and increasing aPCO₂ (table 1). Arterial PO₂ did not change in any of the experiments.

MAP and HR decreased slightly in some of the groups where dorzolamide was injected, but decreased slightly in some of the groups when dorzolamide was injected (table 1).

Injection of timolol did not affect aPH and aPCO₂. However, an injection of dorzolamide created a metabolic acidosis, decreasing aPH and increasing aPCO₂ (table 1). Arterial PO₂ did not change in any of the experiments.

**Carbonic anhydrase inhibitors** may increase ONPO₂ either through a lowering of the intraocular pressure (IOP), a vasodilatory effect on the vessels in the optic nerve, a decrease in the cellular oxygen metabolism, or a combination of these three factors. In this study we used two commonly used glaucoma drugs known to lower the IOP in glaucoma patients.

Pilot experiments have shown that intravenous injections of 100 mg timolol as well as 500 mg dorzolamide in healthy pigs induce similar decreases in IOP of 5–6 mm Hg (data not shown). However, only dorzolamide was able to induce an increase in ONPO₂, demonstrating that not all glaucoma drugs have an effect on the ONPO₂ and indicating that decreasing IOP may not be an important factor for the dorzolamide induced increase in ONPO₂. This is in line with previous experiments in pigs where the effect of dorzolamide on ONPO₂ was shown to be similar in experiments with unclamped and clamped IOP. **Unfortunately, a direct correlation between IOP and ONPO₂ is difficult to show in these types of experiments.** Because the oxygen measurements are invasive, the IOP measurements most likely are affected.

Figure 1 Optic nerve oxygen tension in kPa over time during different kinds of manipulations. (A) The effect of 100 mg timolol injection at time 0 and a subsequent 500 mg dorzolamide injection at 35 minutes. (B) The effect of 500 mg dorzolamide injection at time 0 and a subsequent 100 mg timolol injection at 35 minutes. (C) The effect of a combined injection of 100 mg timolol and 500 mg dorzolamide.

**MAP and HR** decreased slightly in some of the groups where dorzolamide was injected. Dorzolamide is a vasodilatory drug that not only affects the cerebral vessels, but also may affect the systemic arterioles, thereby decreasing the systemic arterial blood pressure. Also the systemic acidosis...
that dorzolamide induces may lower MAP. β Blockers block the adrenergic effect on the vessels and the heart, but timolol affected neither the heart rate nor the mean arterial blood pressure in the pigs in our experiments. However, it is known that β blockers do not cause hypotension in healthy individuals with normal blood pressure. It has been shown that intravenous injections of 0.2 mg timolol in healthy awake humans do not affect mean arterial pressure but do decrease heart rate. In humans the adrenergic positive chronotropic stimulation of the heart is lowered during sleep. We think this is why timolol does not affect the heart rate significantly in the anaesthetised pigs in our experiments.

The experiments were performed with intravenous injections of glaucoma drugs. Dorzolamide was used in a saturating dose of 500 mg and timolol in a comparatively large systemic dose of 100 mg. This application is of course different from the typical topical application of these glaucoma drugs in the human. The aim was to discover the principal pharmacological effects of these drugs on the pig optic nerve and later to proceed to studies with smaller clinical doses in the human glaucoma patient. Having said that, it is of interest that the pharmacological effect of dorzolamide on the optic nerve was present with or without timolol application, before, after, or at the same time as the dorzolamide injection. This suggests that the combination of β blockers and GLAIs may be an effective method of affecting optic nerve oxygen tension and blood flow and, at the same time, lowering IOP.

Authors’ affiliations

J F Kiilgaard, D B Pedersen, M Ia Cour, P K Jensen, Department of Ophthalmology, Rigshospitalet, Copenhagen, Denmark

J F Kiilgaard, D B Pedersen, University of Copenhagen, Denmark

T Eysteinsson, E Stefánsson, Department of Ophthalmology, University of Iceland, Reykjavik, Iceland

K Bang, Merck, Sharp & Dohme, Glostrup, Denmark

Correspondence to: Jens Folke Kiilgaard, MD PhD, Department of Ophthalmology, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen Ø, Denmark; jfk@dadlnet.dk

Accepted for publication 26 May 2003

REFERENCES


Optic nerve oxygen tension: the effects of timolol and dorzolamide

J F Kiilgaard, D B Pedersen, T Eysteinsson, M la Cour, K Bang, P K Jensen and E Stefánsson

*Br J Ophthalmol* 2004 88: 276-279
doi: 10.1136/bjo.2003.021857

Updated information and services can be found at:
http://bjo.bmj.com/content/88/2/276

These include:

**References**
This article cites 15 articles, 5 of which you can access for free at:
http://bjo.bmj.com/content/88/2/276#BIBL

**Email alerting service**
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Topic Collections**
Articles on similar topics can be found in the following collections

- Angle (1006)
- Glaucoma (988)
- Intraocular pressure (1002)

**Notes**

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/