Field potentials of the ganglion cell axons were recorded in the cat from the retinal surface during electrical stimulation of the optic tract. Using this technique during intraocular pressure (IOP) elevation, the impulse conduction was investigated independent of the neuronal input to the retinal ganglion cells. By infusing Na-Nitroprusside intravenously, the mean arterial blood pressure (BPm) of the animal was adjusted to levels between +40 and +135 mmHg. Thus, by setting the perfusion pressure (PP) to values between +30 and -20 mmHg, a large range of IOPs was tested. A PP of +20 mmHg or more left the axonal impulse conduction unimpaired, independent of whether the absolute IOP was 40 or 135 mmHg. Interruption of impulse conduction occurred first at a PP of +10 mmHg. At a PP of 0 mmHg or less, the impulse conduction ceased after a constant time interval (80–120 sec, when 20 Hz electrical stimulation was used). Recovery of the field potentials after restoring normal IOP was independent of the preceding IOP or PP. This data demonstrates that in short-term IOP elevation the electrical function of the ganglion cell axon depends on the PP and not on the absolute height of the IOP. Invest Ophthalmol Vis Sci 24:347–353, 1983.

It is generally accepted that visual field damage in glaucoma is caused by injury to the axons of the retinal ganglion cells. The mechanisms that lead to degeneration, however, are still under discussion. Both mechanical alterations and vascular insufficiency have been incriminated and were proven to result from intraocular pressure (IOP) elevation. Recent experimental studies with short- and long-term IOP elevation showed the pattern of degeneration, interruption of axoplasmic transport, and a reduced filling of the prelaminar capillaries of the optic nerve head.

The axoplasmic transport depends on the absolute height of the IOP. This can be concluded from an experiment in which the axoplasmic transport was blocked by increasing the IOP even though the oxygen supply was maintained by hyperbaric O₂ respiration. On the other hand, the electrical function of the retina depends on the oxygen supply. This has been shown by two types of experiments. First, decreasing the perfusion pressure (PP) by lowering the blood pressure and keeping the IOP normal abolishes the signal transmission to the optic nerve. Second, the ERG b-wave, being extinguished during IOP elevation, is restored by hyperbaric oxygen respiration of the experimental animal. In both experiments, a light stimulus was used, and the responses depended not only on the function of the cells from which the record was taken but also on the function of the preceding neurons.

Relevant for the problem of glaucoma, however, is the specific sensitivity of the function of the ganglion cell axons. The present study uses a stimulation and recording technique that allows for determination of the electrical function of the axons independent of the neuronal input to the retinal ganglion cells. By testing the sensitivity of the retinal nerve fibers at various arterial blood pressures and intraocular pressures, it could be determined whether the electrical function of the axon depended on the absolute height of the intraocular pressure, or on the perfusion pressure.

Materials and Methods

The experiments were performed on eight adult cats of normal weight (2–5 kg), which were anesthetized intraperitoneally with a dose of 30 mg/kg pentobarbital-Na and 0.5 mg of atropine. General anesthesia was maintained by injections of 5 mg/kg pentobarbital-Na through a venous catheter every 4 hrs.

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Local infiltration anesthesia was performed with me-pivacain 1% (3–5 ml) at the supra- and infra-orbital branches of the trigeminal nerve. The cat was mounted in a stereotactic head holder.

The eye was fixed by a Flieringa ring that was firmly connected to the stereotactic apparatus. The sclera was exposed, and the lateral orbital tissues were removed. A scleral hole was cauterized 4–5 mm behind the limbus, and a guiding tube was inserted. For recording, a tungsten electrode coated with polyvinylchloride was used. Its diameter fitted snugly into the guiding tube in order to prevent leakage of intraocular fluid. The position of the electrode tip on the retina was adjusted by a microdrive during indirect binocular ophthalmoscopy.

A bipolar stimulating electrode was stereotactically inserted into the optic tract ipsilateral to the eye under investigation. Its position was adjusted according to the evoked cortical potentials that were recorded from the occipital lobe during electrical stimulation. In order to elicit an antidromic field potential of the nerve fiber layer of the retina, repetitive suprathreshold stimuli (5–20 V) of 0.3 msec were applied to the optic tract.

A stimulation frequency of 20 Hz was chosen in order to simulate an average discharge rate within the physiological range. Figure 1 gives a schematic representation of the experimental set-up.

The arterial blood pressure was measured continuously through a femoral catheter inserted into the abdominal aorta. The mean arterial blood pressure (BPm) was calculated as follows: diastolic BP + (systolic BP – diastolic BP)/2. The BPm normally was 100–130 mmHg at the beginning of the experiment. One cat had a BPm of 160 mmHg. By infusion of Na-Nitroprusside (0.12–0.36 mg/kg/hr), the BPm could be maintained artificially at stable lower levels during the recording periods. By stopping the infusion of Na-Nitroprusside the pressure would increase and stabilize within a few minutes. The BPm levels during a particular recording session were chosen at random.

The IOP was varied artificially from 25 to 145 mmHg by adjusting a Ringer solution reservoir to the appropriate height. The Ringer solution contained 20 units/ml heparine in order to prevent clotting. The reservoir was connected to a cannula that was inserted into the anterior chamber of the eye. The actual IOP was measured separately through a second cannula by a Statham pressure transducer. The PP was calculated as the difference between the mean arterial blood pressure and the intraocular pressure (PP = BPm – IOP). At each BPm level, PPs of +30, +20, +10, 0, −10, and −20 mmHg were tested, and the amplitude of the nerve fiber potential was measured as a function of time (Fig. 2). Since it was difficult to determine when the amplitude of the potential reached zero, we chose a time, T20, when the potential decreased to 20% of its initial value.

When the IOP was raised abruptly to high values, the immediate distension of the globe resulted in a moderate reduction of the signal amplitude. This was due to a movement of the retinal surface away from

![Fig. 1. Schematic representation of stimulation and recording sites for testing the sensitivity of the nerve fibers in the cat eye (left eye seen from above). Elevation of IOP was produced by the height of a Ringer solution reservoir, connected to the anterior chamber of the eye. The IOP was measured separately. Left upper insert: typical field potential obtained from the fast (I) and the slow (II) conducting nerve fiber group.](image-url)

![Fig. 2. Time course of the decay of the potential amplitudes (I and II) after the IOP was suddenly raised to the BPm (PP = 0). BPm = 75 mmHg, IOP = 75 mmHg. The residual conduction time until the amplitude decreased to 20% of its plateau value is named T20 (sec). Smaller axons • A (II) are influenced somewhat earlier than larger ones • B (I). Recovery is given in an extended time scale.](image-url)
the electrode. When the IOP was set to 100 mmHg, this movement of the eye wall was approximately 150 μm that could be measured by advancing the electrode immediately after the pressure step with the microdrive. However, advancing the electrode is not desirable because a subsequent pressure release would cause retinal damage by movement of the retinal surface towards the electrode. On the other hand, it was not necessary to advance the electrode after a pressure step because the moderately reduced amplitude could be taken as the new baseline. This new baseline was reached within 5 to 10 sec, and it did not interfere with the later collapse of the amplitude caused by the pressure ischemia (Fig. 2).

All data were stored on an FM magnetic tape and later analysed.

Results

Figure 1 (left upper insert) shows the field potentials from the retinal surface during antidromic electrical stimulation of the optic tract. When the electrode tip was positioned between the disc and the area centralis of the retina, the field potentials originated exclusively from the axons during ipsilateral optic tract stimulation. The recorded potential consisted of two sharp peaks (I and II), representing two types of ganglion cell axons that differ in conduction velocity. These two conduction velocity groups correspond to the physiologic classification of Y- and X-ganglion cells.13,16,17 W-cells cannot be recorded with this method routinely, because their conduction velocities are more scattered and, therefore, do not give rise to a distinct peak in the recorded potential.

A first impairment of impulse conduction during IOP elevation occurred when the IOP was set to 10 mmHg below the mean arterial blood pressure (BPm). At 20 mmHg below the BPm, with very few exceptions, the impulse conduction was unimpaired. When the retinal circulation was interrupted by very high intraocular pressure (IOP > BPm), the time course of the amplitude collapse was nearly constant.

Figure 2 shows the time course of the fast (I) and the slow (II) conducting axon potential amplitude after abrupt elevation of the IOP to the BPm (PP = 0). After an initial drop of the amplitude due to mechanical distension of the globe, a plateau was reached. Fifty seconds later, a decrease of the potential amplitude began progressing to a complete interruption of impulse conduction after 100–120 seconds. The impulse conduction in the smaller axons (II) was interrupted earlier than in the larger ones (I).

In order to determine whether the absolute IOP or the PP was responsible for the interruption of the impulse conduction, the blood pressure had to be varied over a wide range, and different IOPs had to be applied at each blood pressure level. A steady reduction in blood pressure was achieved by intravenous infusion of Na-Nitroprusside. Na-Nitroprusside by itself did not alter the nerve fiber potential as long as the BPm was maintained at 50 mmHg or more and the IOP was normal.

For each blood pressure level, PPs of +30, +20, +10, 0, −10, and −20 mmHg were tested in a random sequence. Hence, a range of IOPs between 25 and 145 mmHg was covered. Figure 3 shows all recorded IOP/BPm pairs and gives qualitative information as to whether the impulse conduction was unimpaired (0) or interrupted (+). One can clearly see the border between unimpaired and interrupted impulse conduction at 10 to 20 mmHg below the BPm (broken line at PP = +15 mmHg). With a BPm of 55 mmHg, the conduction of impulses was interrupted at an IOP of 45 mmHg, whereas an IOP of 140 mmHg did not impair the impulse conduction in one animal in which the blood pressure was 160 mmHg. From this data it is clear that the sensitivity of the impulse conduction depended on the PP and not on the absolute IOP.

In order to rule out an increase of the sensitivity of the axons with the duration of the experiment, all data were also evaluated regarding the point of time...
Fig. 4. Residual conduction time $T_{20}$ of retinal axons as a function of perfusion pressure. The values are classified in arbitrary blood pressure ranges: A, 50–70 mmHg; B, 70–90 mmHg; C, 90–110 mmHg; D, 110–130 mmHg. • • fast conducting fiber group (I). • • slow conducting fiber group (II).

at which they were obtained, but no correlation could be found. Hence, it can be concluded that the vital functions of the animal were stable and did not interfere with the tested parameters.

In order to quantify the sensitivity of the axonal impulse conduction, the interval between the onset of the IOP increase and the reduction of the amplitude to 20% of the plateau value ($T_{20}$) was measured (Fig. 2). These $T_{20}$-values are shown in Figures 4A–D as a function of the perfusion pressure for an arbitrary classification of the blood pressures. No decrease in the amplitude occurred in nearly all recordings when the PP was +20 mmHg or greater. At a PP of +10 mmHg, the nerve fiber potential was extinguished within 180–300 sec. The large standard deviation at the PP of +10 mmHg is due to the steep decrease of the curve in this section where minor variations of BPm values caused considerable prolongation or shortening of the residual conduction time $T_{20}$. When the PP was set between 0 and −20 mmHg, $T_{20}$ was about constant (80–120 sec). Comparison of Figures 4 A–D shows that all curves are of similar shape, although the IOPs necessary to produce the perfusion pressure levels differed greatly.

Statistical evaluation of the data revealed that there was a correlation ($P < 0.05$) between the PP and the interruption of the impulse conduction in each animal, whereas there was no correlation between the absolute IOP and the interruption of impulse conduction.

After reduction of the IOP to normal, the recovery of the axonal conduction was complete within 30 sec when the period of the preceding ischemia lasted 5 min. For both groups of axons (I and II) the time course of recovery was similar. Figure 5 gives the mean values of the amplitude recovery curves, classified according to the preceding PP (A) or BPm (B). Figure 5A shows that the average time course of the recovery of the potential was similar irrespective of whether the PP that led to interruption was +10, 0, −10, or −20 mmHg. When the data was classified according to the blood pressures (Fig. 5B), all axons that recovered from ischemia at higher BPm levels showed regular impulse conduction within 30 sec (BPm = 70–130 mmHg). The recovery during experiments performed at a BPm of 55 mmHg was slower than the others. It can be inferred from Figure 5B that preceding high IOPs do not delay the recovery, for high blood pressures required the application of high IOPs and, vice versa, low blood pressures required only relative low IOPs for interruption of impulse conduction. This argues against a mechanical influence in our experiments and supports the view that the restitution of the oxygen supply is the important factor for recovery of axonal conduction after short-term IOP elevation.

**Discussion**

The present study was undertaken to quantify the pressure sensitivity of impulse conduction in the ganglion cell axons of the retina. Our data demonstrates that during acute short-term intraocular pressure (IOP) elevation, maintenance of the impulse conduction depends on the arterial perfusion pressure (PP) of the eye and not on the absolute IOP. By lowering the arterial blood pressure with Na-Nitroprusside, critical PPs were tested at various IOP levels.

As long as the PP was +20 mmHg or more, the impulse propagation in the axons was normal. It is remarkable that short-term IOP elevation up to 140 mmHg did not alter the impulse conduction by me-
Mechanically stretching or kinking the axons as long as an adequate PP of \( \geq +20 \text{ mmHg} \) was maintained (Fig. 3). At a PP of \(+10 \text{ mmHg}\), the conduction of impulses along the ganglion cell axon was critically influenced, and at a PP of \(0 \text{ mmHg}\) or less, the impulse conduction was interrupted after a constant time interval (Figs. 2, 4). This residual conduction time after arresting the ocular circulation probably depends on the reservoirs of ATP \(^{18}\) or intra-axonal potassium, \(^{19}\) which allow a certain number of impulses to be transmitted after the onset of total ischemia. Hence, when the stimulation frequency is increased, the residual conduction time is shortened and vice versa. \(^{19}\) Small axons, having less intra-axonal potassium, cease somewhat earlier in their ability to conduct action potentials than do the larger ones (Figs. 2, 4).

Impulse conduction after blockade by pressure ischemia showed a uniform course of recovery irrespective of the preceding IOP values. However, the recovery was delayed when the aortic blood pressure was very low (55 mmHg). Other experiments showed that the recovery was also delayed when the duration of the preceding ischemia was prolonged, \(^{20,21}\) or when repetitive IOP elevations with short intervals were applied. \(^{19}\)

These results support the suggestion that the blood supply plays an important role in the maintenance of retinal nerve fiber function during short-term IOP elevation. One must consider, however, that the oxygen supply to the inner retina is not linearly related to the calculated perfusion pressure because autoregulative mechanisms are present in the retinal circulation of the cat similar to the primate. \(^{22-24}\) When the IOP of the eye is increased, no significant change in retinal blood flow occurs until the PP is decreased to \(50 \text{ mmHg}\). At this level, the maintained activity of the retinal ganglion cells begins to change. \(^{10,22}\) The axons, however, which in our study were tested independently from the other retinal neurons, change their conduction properties only at PPs below \(+20 \text{ mmHg}\). According to the data of Flower and Patz, \(^{12}\) the blood flow of the retinal arteries is then reduced to 20–30%. The cessation of blood flow occurs at a PP of \(< +10 \text{ mmHg}\). Hence, a considerable degree of ischemia is tolerated by the membrane processes of the axons.

In previous studies, the oxygen dependence of the neuronal function was shown in various elements of the retina during short-term IOP elevation, \(^{25-28}\) during arterial hypotony, \(^{10,11}\) and during hypoxia. \(^{30-34}\) However, for the problem of glaucoma, the isolated function of the ganglion cell axons should be investigated. Therefore, the evaluation of the ERG is not a suitable approach, because it is generated by neuronal elements distal to the retinal ganglion cells. \(^{35,36}\) Similarly, single cell responses \(^{25,37,38}\) or mass potentials from the optic tract, \(^{11,27-29}\) elicited by light stimulation, do not give specific information about the axons because ischemia also influences all retinal neurons that contribute to the input of the retinal ganglion cells. Therefore, the alteration of the neuronal response during light stimulation will be dominated by the weakest link within the retinal neuronal network. It was suggested that during acute oxygen deprivation the weakest link lies between the receptors and the retinal ganglion cells. \(^{25,30,32,39}\) This view was supported by an experiment in which 9% oxygen respiration of
the animal left the axonal impulse conduction unimpaired, whereas the simultaneously recorded ERG b-wave was completely suppressed.14 Therefore, during graded short-term oxygen deprivation, the electrical function of the axon is less sensitive than are the synaptic processes of the retina. Noell30 has come to a similar conclusion, but he tested the myelinated part of the axon between the optic nerve and the optic tract and compared it to the ERG. The conduction properties of the myelinated segments are certainly more stable than those of the unmyelinated ones. In addition, only the latter are directly affected by intraocular pressure. Therefore, in our study, the technique of antidromic stimulation and IOP elevation was chosen to “isolate” functional changes of the intraocular unmyelinated part of the ganglion cell axons.

We do not think that the different anatomy of the vasculature of the optic nerve head in the cat limits the application of our results to the situation in primates. The retinal circulation of the cat differs from that in the primate in that the retinal arteries are fed by ciliary vessels that get the blood from the external carotid artery via the internal maxillary artery.40,41 However, in both cats and primates, the nerve fiber layer of the retina is nourished exclusively from the retinal arteries,42 which in both species furnish a superficial and a deep capillary net of comparable microarchitecture40 and functional characteristics.23,24 In the present study we only tested the sensitivity of the axons during short-term IOP elevation. Therefore, the condition of the experiment corresponds particularly to the situation of acute angle closure glaucoma. The relatively high resistance of axons to short-term IOP elevation may be an explanation why glaucomatous cupping does not occur after acute angle closure glaucoma.43 During long-term IOP elevation, however, the sensitivity of impulse conduction of the axons may be different. Moreover, in chronic open-angle glaucoma, not only ischemia but also structural changes at the lamina cribrosa44 and mechanical interruption of axoplasmic transport3,6 are probably involved in the process of axon bundle degeneration.

Key words: intracocular pressure, perfusion pressure, retinal nerve fibers, axonal conduction, electrical stimulation, cat, Na-Nitroprusside

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