Neuroprotection in glaucoma-electrophysiology (Review)

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Abstract. Hypertensive glaucoma is defined as a group of diseases with progressive loss of the neuroretinal margin of the optic disc that causes characteristic degenerative optic neuropathy. The present study provided an updated summary of the physiology and pathology of neurotransmission in the visual path, with the focus on glaucoma. The results of positron emission tomography, functional magnetic resonance imaging and mainly electrophysiological methods demonstrated pathogenesis of nerve cell damage in the visual pathway. Based on these conclusions, neuroprotection in glaucoma was proposed. This consists mainly of the reduction of the intraocular pressure. It is followed by a decrease of glutamate in the synaptic cleft and blockade of its binding to the NMDA receptors. The supply of energy substrates to altered nerve cells is also indispensable. Therapy should be systemic due to impairment of the complete visual path.

Contents

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Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-8 isoxazolpropionate; c/d, cup-to-disc diameter ratio; EEAT, excitatory amino acid transporter; fMRI, functional magnetic resonance imaging; GLAST, glutamate aspartate transporter; GS, glutamine synthetase; IOP, intraocular pressure; NMDA, N-methyl-D-aspartate; MC, Muller cells; PERG, pattern elektroretinogram; PVEP, pattern visual evoked potential

Key words: physiology and pathology of transfer in the visual path, glaucoma, neuroprotection

1. Introduction

Glaucoma is defined as a group of diseases with progressive loss of the neuroretinal margin of the optic disc that causes characteristic degenerative optic neuropathy (1). There is a sufficient number of studies in the literature focusing on the topic of neuroprotection in glaucoma (2-5). Therefore, we will not deal with the issue of antioxidants, adenosine receptor antagonists, nicotinic acetylcholine agonists, neurotrophic factors, metabolic products in ganglion cell necrosis and apoptosis, etc.

2. Electroretinogram and visual evoked potentials

One of the first stimuli that led us to the study of glaucoma was the simultaneous measurement of the pattern electroretinogram (PERG) and pattern visual evoked potential (PVEP) in a 20-year-old healthy individual. At first, the intraocular pressure (IOP) was 15mmHg and it subsequently increased to 40 mmHg. Surprisingly, neurotransmission was blocked at the level of the retinal ganglion cell level, while PVEP changed slightly (Fig. 1). This fact did not correspond to the existing definitions of glaucoma regarding impairment of the retinal ganglion cell axons with excavation on the optic disc and changes in the visual field. With the blockade of transport at the level of the ganglion cells, we expected the absence of, or at least abnormal PVEP response. Measurements were taken in 1987 (6).

Therefore, we searched for an answer to this response of the visual analyser. Several questions remained unanswered. Why did the retinal ganglion cells not respond and what happened to the central visual pathway, when we get an almost normal response following the blockade at the level of the retinal ganglion cells in the brain? What is the reason for us not noticing the first changes at the level of the axons of the retinal ganglion cell, when all the previously available glaucoma definitions indicated this? There is one explanation for an electrophysiologist. Following the stabilisation of the binocular functions, the visual cortex is set up to receive a certain amount of action potentials. When it is decreased at any level from the photoreceptors to the cortical cells, it starts to use the feedback processes to determine at which level this lesion occurred (7-10).

Before explaining the above mechanisms, I need to briefly explain the process of transmission of the electrical changes in the visual pathway, from the photoreceptors to the visual centres of the brain.

3. Transmission of the electrical changes in visual pathway

Following the impact of light on the retina, a chemical change occurs in the outer photoreceptor segments (cis-retinal is changed to trans-form). This causes their hyperpolarisation (11).

Hyperpolarisation of the photoreceptors during the synaptic transfer causes a release of glutamate from the presynaptic neuron into the synaptic cleft and subsequent binding to the receptors located on the membrane of the post-synaptic neuron (12).

Glutamate is bound to the receptors which were named based on their selective agonists. N-methyl-D-aspartate is a typical agonist for the NMDA receptors. A typical agonist for the AMPA receptors is α-amino-3-hydroxy-5-methyl-4-8 isoxazolpropionate (AMPA), and for the third type, kainate receptors, kainate. The AMPA and kainate receptors are also called non-NMDA (13).

The NMDA receptors represent ion channels permeable for calcium (Ca) ions. Calcium flow through the NMDA receptors is blocked by the magnesium (Mg) ions at a normal membrane potential. This block can be eliminated by strong depolarisation (14).

Excessive calcium influx into the cells through the NMDA voltage-gated channel can be caused by hypoxia, hypoglycaemia, etc. Under these conditions, the level of glutamate in the synaptic cleft remains elevated for a long time, with sustained activation of the NMDA receptors, resulting in such intracellular calcium concentrations that are cytotoxic. Inhibition of the NMDA receptors can delay this dying, using their antagonists (15).

Concentration of free glutamate in the synaptic cleft achieves approximately 1.1 mM during the synaptic transfer. However, its concentration quickly drops and it breaks down in the NMDA receptors during 1.2 ms. However, glutamate is dissociated much faster from the AMPA receptors. Thus, the time course of free glutamate predicts that dissociation contributes to the breakdown of the postsynaptic flow mediated by the AMPA receptors. Otherwise, the voltage-gated channels would open (12).

Glutamate in the mammalian central nervous system is eliminated from the synapsis mainly by the glutamate transporters of the excitatory amino acid transporter type (EEAT) and glutamate aspartate transporter (GLAST) as glutamate transporter to the Muller cells (MC) and glutamine synthetase (GS) as glutamate to glutamine in MC (16,17) (Fig. 2).

Subsequently, in glial cells, glutamate is converted to glutamine, which no longer acts as a neurotransmitter and can thus be released back into the synapsis, from where it is subsequently taken up by the presynaptic neuron, which converts it back to glutamate (18).

To date, there is no evidence of the presence of an enzyme that would convert glutamate directly in the synapsis (19).

All of the above is to explain the processes involved in the transmission of the electrical voltage changes in the visual pathway.

4. Restoring of action potentials

We have two possibilities to recover the amount of action potentials coming to the brain to the baseline values. The first is the release of a greater amount of neurotransmitter at the level of the 'damaged' cell, and the second is to keep this neurotransmitter in the synaptic cleft for a longer period. Both possibilities were experimentally proven in glaucoma.

In the vitreous humour of the glaucoma eyes of experimental animals, the glutamate (27 μM) value was up to 3-fold higher compared to the control group. These values are toxic both for the ganglion cell layer and for the internal plexiform layer (20).

The GLAST and GS values were increased after just 3 weeks, following the increase of the IOP in rats. The number of ganglion cells was decreased to 6 and 44% after 4-60 weeks from the increase of IOP, respectively (21). Glutamate receptors are expressed not only in the retinal ganglion cells, but also in the photoreceptors, as well as in the horizontal and bipolar cells (22).

The long-term effect of glutamate on the non-NMDA receptors increases the postsynaptic potential and opens the voltage-gated receptors that are normally closed by magnesium and the entry of calcium into the cell. This process takes places in all cells which have glutamate receptors. Therefore, not only the retinal ganglion cells are impaired, but also the cells in the internal core layer and the layer of photoreceptors (23).

The question remains why the signal transduction failure occurred at the level of the retinal ganglion cells. We found the explanation in the study by Shou *et al* (24). They qualitatively studied alpha and beta retinal ganglion cells following acute increase of IOP. The analysis found that cell density, size of the body, maximum diameter of the dendritic field, total dendritic length and the number of branches of dendritic bifurcations were significantly decreased in the glaucoma eyes, compared to the healthy group. Loss of cells and shrinking of dendrites in the type alpha retinal ganglion cells were more pronounced compared to the beta cells. The density of all types of retinal cells and corpus geniculatum laterale declined over time if the IOP was increased, and the loss of cells was more significant in large cells (alpha) compared to small cells (beta). Ischaemia has a major influence on the decrease of the dendritic diameter and cells alone. Larger ganglion cells are more sensitive to the environmental changes (ischaemia) because of their energy performance (24).

The nerve cells do not die immediately following the influx of calcium to the cells. As stated above, their size is first reduced. If they have a sufficient energetic reserve, they will cope with this state. As soon as the energy is depleted, the apoptotic or necrotic process is initiated and the cell dies.

5. Damage of the visual brain centres

If the visual pathway, including the visual cortex, is involved in the process of hypertensive glaucoma, then we should also find changes in the brain. The standard structural examination techniques do not make this diagnosis possible. For this reason, we used positron emission tomography. Radioactive glucose (18 fluorodeoxyglucose), which is taken up in healthy cells, is used to examine brain activity. Fig. 3 shows the absence of glucose radioactivity in the area of the occipital lobe. The examination was performed in 2001 (25). Visual field and image of functional magnetic resonance imaging (fMRI) are shown in Figs. 4 and 5. For comparison, we also present the normal fMRI findings in a female patient with normotensive glaucoma (Fig. 6). Using positron emission tomography and

fMRI, we found that damage of the visual brain centres occurs in hypertensive glaucoma as well.

6. Determining the level and depth of damage

During the experimental glaucoma, the electroretinographic changes (decrease of the amplitudes by up to 50%) preceded the changes in the retinal neuronal fibre layer (26). These facts, as well as the conclusions of other authors (9,24,26), forced us to use electrophysiological methods (PERG and PVEP) to determine the level and depth of damage in various types of hypertensive glaucoma (27-29). Based on these examinations regarding the changes in PERG and PVEP, we concluded that glaucoma causes damage not only to retinal ganglion cells and subsequently their axons, but also to the visual centres in the brain (30).

At the level of neuronal membrane, the mutual relationship of both neurotransmitter systems is documented by direct inhibition of the NMDA receptor by dopamine and the inhibitory effect of glutamate on the release of dopamine. This means that a higher level of dopamine blocks the NMDA receptor and, conversely, glutamate blocks the release of dopamine (31,32).

We used the examination of the oscillation potentials of the electroretinogram for verification of this biochemical information. Amacrine cells are divided into dopaminergic and GABAergic, based on the neurotransmitter. Dopaminergic cells generate oscillation potentials in the electroretinogram and GABAergic cells take part in the development of the threshold scotopic potential (33).

We performed the examinations in 2001, using the Primus (Lacce Elettronica) device according to the ISCEV methodology (1989). Following a 30-min adaptation to dark, we examined the oscillation potentials. Stimulation of the retina during artificial mydriasis (0, 5% tropicamid) was performed, using the flashlight of 5 ms in length and luminous flux of 2.5 cd/m^2/s. Ten responses (stimuli in 15-sec intervals) were averaged, using filters from 80 to 500 Hz. We evaluated the latency and amplitude of the P2 oscillation.

The first group consisted of 23 eyes of healthy people. In the second group, there were 36 glaucoma eyes with imminent changes in the visual field with compensated IOP. The mean age of the persons included in the groups was 40.3 years (range, 35-56). The results showed a significant prolongation of latency of the P2 oscillation (P=0.049) and a decrease of the oscillation amplitude (P=0.001) in glaucoma eyes, compared to the healthy group.

We demonstrated indirectly increased values of glutamate in the glaucoma eyes. We were also interested in how the retinal ganglion cells (PERG) would behave during modification of the anti-glaucoma therapy and subsequently the complete visual analyser (PVEP).

We performed the PERG and PVEP examination (using the ISCEV methodology-2012 on the Roland Consult SRN device) in a female patient (64 years) with glaucoma, compensated with dorzolamid, timolol meleas and brominidin to the IOP of 18/18 mmHg. The visual field was within the normal ranges, c/d=0.4 and nerve fiber layer index 11/20 (normal values). With regard to these values, we repeated the examination in one month, following discontinuation of both anti-glaucoma medications. IOP was increased to 23/29 mmHg. The PERG amplitude P50 and N95 was reduced by 3.2/1.1 μV following discontinuation of the medication (Fig. 7) and, on the contrary, it was increased in PVEP by 1.4/4.7 μV (Fig. 8).

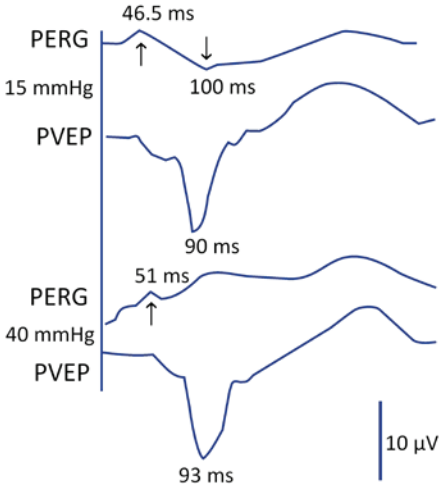

Figure 1. Upper curve (PERG) and below it the PVEP curve at normal IOP. The arrows indicate latency of the oscillations whose amplitude occurs as a response of the retinal ganglion cells. The lower curves show the situation following increase of the IOP to 40 mmHg (6). IOP, intraocular pressure; PERG, pattern elektroretinogram; PVEP, pattern visual evoked potential.

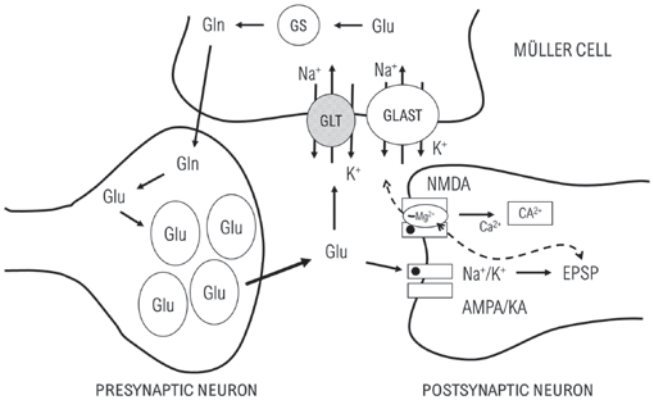

Figure 2. Signal transmission in the visual pathway. Glu, glutamate; Gln, glutamine; GS, glutamine synthetase; AMPA, α-amino-3-hydroxy-5-methy l-4-isoxazolepropionic acid; KA, kainic acid; EPSP, excitatory postsynaptic potential; NMDA, N-Methyl-d-aspartate; GLAST, glutamate aspartate transporter.

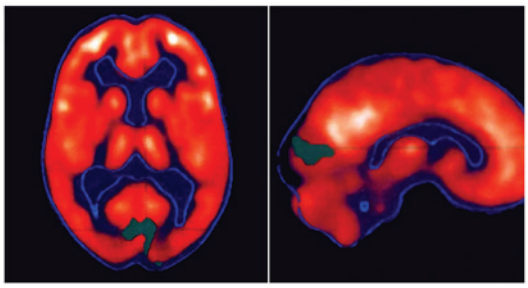

Figure 3. Sagittal section through the brain of a 58-year old patient with secondary glaucoma. Visual acuity of the right eye, 0.05, light projection is correct. Visual acuity of the left eye, 1.0 neutral. c/d=1.0. Green colour demonstrates a deficit of fluorodeoxyglucose in the visual centre (25). c/d, cup-to-disc diameter ratio.

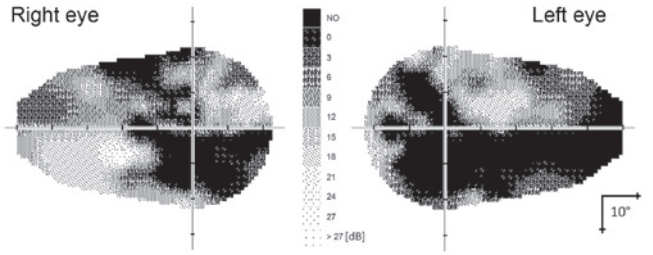

Figure 4. Visual field of the same patient as in Fig. 3 from the same period of time.

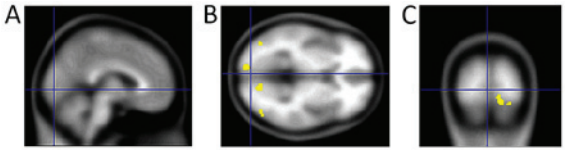

Figure 5. Functional magnetic resonance imaging following visual paradigm, using black and white chessboard in the same patient as in Fig. 3. The examination was performed in 2010. Yellow areas indicate increased activity. (A) Sagittal section, (B) transverse section and (C) coronal section based on Lestak *et al* (25).

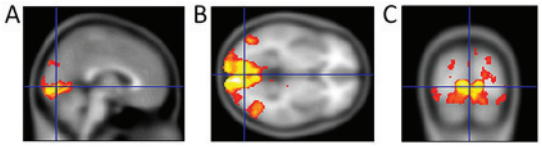

Figure 6. Functional magnetic resonance imaging in a patient with normotensive glaucoma (age, 61 years). V, 1,0 without correction. Cup-to-disc diameter ratio, 1 (25). Yellow areas indicate increased activity. (A) Sagittal section, (B) transverse section and (C) coronal section.

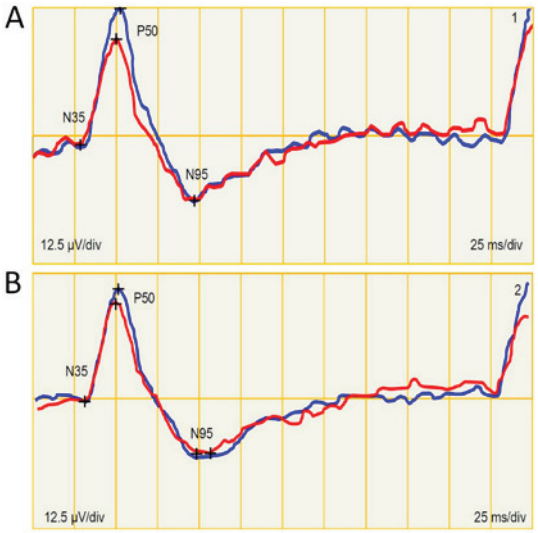

Figure 7. Pattern elektroretinogram before discontinuation (blue line) and after discontinuation of anti-glaucoma medicines (red line). (A) Right eye; (B) left eye.

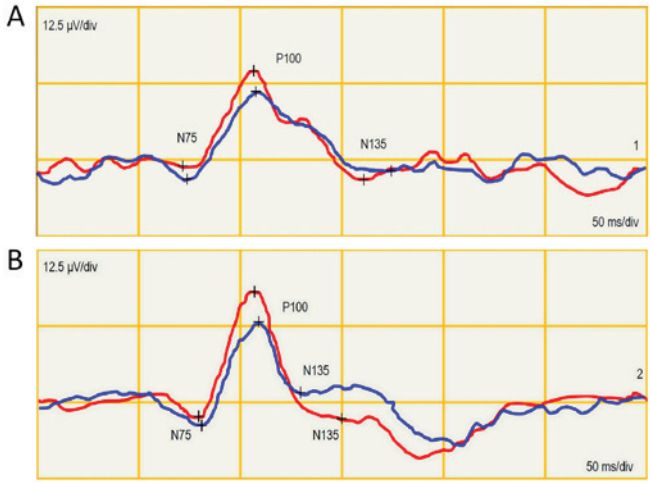

Figure 8. Pattern visual evoked potential before discontinuation (blue line) and after discontinuation of anti-glaucoma medicines (red line). Following stimulation of the (A) right eye and (B) left eye.

This finding also shows alteration of the retinal ganglion cells and, on the contrary, potentiation of the visual pathway with glutamate.

Because, even with IOP controlled, the number of action potentials is reduced due to the loss of cells involved in processing of the electrical changes in the visual pathway, these cells are 'bombarded' to a higher response by the feedback mechanisms. Excessive release and decreased absorption of glutamate from the synaptic cleft result in increase of the postsynaptic potential. Subsequently, the voltage-gated channels are then unblocked for influx of calcium into the cells and the whole process progresses. With disease progression, the response to releasing more neurotransmitter is also higher.

We also confirmed this in the study in which we observed progression of changes in the visual fields in the compensated glaucoma eyes in a five-year period. We found that the bigger the initial perimetric changes were, the bigger was their progression in five years (34).

Electrophysiological methods are not commonly used to diagnose glaucoma. They are used in our clinic in questionable cases, but mainly to verify normotensive glaucoma. Based on this knowledge, neuroprotective therapy may be offered. We put a decrease of the IOP first. This is followed by a decrease of glutamate in the synaptic cleft and a blockade of its binding to the NMDA receptors. Supply of the energy substrates to altered nerve cells is also indispensable. Local ophthalmologic treatment will not affect subcortical and cortical visual centers. Neuroprotective treatment should be systemic because of impairment of the complete visual pathway. However, attention should be drawn to the side effect of NMDA receptor antagonists, which induce symptoms like schizophrenia (35).

7. Conclusion

Impairment of the whole visual pathway occurs in hypertensive glaucoma. Therefore, early diagnosis of the disease is important. Treatment should be based not only on a reduction of the IOP, but also on a decrease in glutamate levels in the synaptic cleft and their binding to glutamate receptors. Delivery of the energy substrate to the nerve cells, with the possibility of dealing with the intracellular processes is an important part of therapy. This therapy should be systemic.

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Availability of data and materials

All datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JL is the author of the main idea, and designed and created the main theoretical parts of this review. MF contributed to the design and implementation of research, examination image results analysis and to writing of the manuscript. JL explained the ophthalmological and electrophysiological context.

Ethics approval and consent to participate

All patient results and images included in this review were retrospectively used with prior patient consent. The consent was in accordance with the principles stated in the Helsinki Declaration and as approved by the Internal Ethics Committee of the Eye Clinic JL Faculty of Biomedical Engineering CTU in Prague.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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