Glaucoma and Alzheimer: Neurodegenerative disorders show an adrenergic dysbalance

Bettina Hohberger^{1*}, Harald Prüss², Christian Mardin¹, Robert Lämmer¹, Johannes Müller³, Gerd Wallukat³

¹ Department of Ophthalmology, Friedrich-Alexander-University of Erlangen-Nuremberg, Erlangen, Germany

² Department of Neurology, Charité'-Universitätsmedizin Berlin, Berlin, Germany

³ Berlin Cures GmbH, Berlin, Germany

* Corresponding author

Corresponding author:

PD Dr. med. Dr. rer. biol. hum. Bettina Hohberger

Department of Ophthalmology

University of Erlangen-Nürnberg

Friedrich-Alexander University of Erlangen- Nürnberg

Schwabachanlage 6

91054 Erlangen

phone:+49 9131-853301

e-mail: bettina.hohberger@uk-erlangen.de

Keywords: autoantibodies; glaucoma; alzheimer; β 2-adrenergic receptor; imbalanced autonomic theory; amyloid beta.

Abstract

Glaucoma disease is characterized by an increased intraocular pressure (IOP), glaucomatous alterations of the optic disc and corresponding visual field defects. Even lowering the main risk factor IOP until an individual target level does not prevent this neurodegenerative disorder from proceeding. Several autoimmune mechanisms were discovered, partly showing a functionality. One of these autoimmune phenomena targets the $\beta 2$ adrenergic receptor ($\beta 2$ -AR; i.e. agonistic autoantibodies; $\beta 2$ -agAAb) and is linked to the elevated IOP and an impaired retinal microcirculation. As neurodegenerative disorder, Alzheimer's Disease (AD) is postulated to share a common molecular mechanism with glaucoma. In the present study we investigated autoimmune phenomena targeting the $\beta 2$ -AR in patients with AD. Sera of the patients were analyzed in a rat cardiomyocyte bioassay for the presence of functional autoantibodies against $\beta 2$ -AR. In addition, different species of amyloid beta (A β) monomers were tested (A β 1-14, A β 10-25, A β 10-37 A β 1-40, A β 1-42, A β 28-40, and [Pyr]-A β 3-42). Our results demonstrate that none of the short-chain A β (A β 1-14, A β 10-25, or A β 28-40) showed any agonistic or inhibitory effect on $\beta 2$ -AR. Contrary, long-chain [Pyr]-A β 3-42, representing a major neurogenic plaque component, exerted an activation that was blocked by the $\beta 2$ -AR antagonist ICI118.551 indicating that the effect was realized via the $\beta 2$ -AR. Moreover, the long chain A β 1-40, A β 1-42, and A β 10-37 yet not the short-chain A β peptides prevented the clenbuterol induced desensitization of the $\beta 2$ -AR. In addition, we identified functional autoantibodies in the sera of AD patients, activating the $\beta 2$ -AR like the $\beta 2$ -agAAb found in patients with glaucoma. As autoimmune mechanisms were reportedly involved in the pathogenesis of glaucoma and Alzheimer's Disease, we postulate that overstimulation of the $\beta 2$ -AR pathway can induce an adrenergic overdrive, that may play an important role in the multifactorial interplay of neurodegenerative disorders.

Introduction

Glaucoma disease is a worldwide burden, being the second leading cause of blindness in the industrial nations. This neurodegenerative disease is characterized by an increased intraocular pressure (IOP), a glaucomatous optic disc and visual field defects, going along with disease progression. Up to now the exact pathomechanisms are still elusive. An elevated IOP, the main risk factor, is the target of conservative, laser and surgical therapy. Yet, despite lowering IOP, glaucoma disease proceeds, as no causal therapy is available until now. Neurodegeneration in glaucoma is featured by apoptosis of the retinal ganglion cells (RGC). Immune and autoimmune mechanisms can interplay in this molecular interactions as recent studies suggested. Data of an animal model (mice) yielded a significant RGC loss mediated by T-lymphocytes via heat-shock-proteins in the presence of only less elevated IOP. (Chen et al., 2018) Even normotensive eyes were observed to show an apoptosis of RGC when the contralateral eye was hypertensive (mice model). This effect was attributed to lymphocytes as well. (Gramlich et al., 2020) Apoptosis itself, a vascular dysregulation or other external factors are able to trigger immune and autoimmune mechanisms. Thus, it is not surprising that diverse autoantibodies were detected in sera of patients with glaucoma. In this context, autoantibodies with a functional consequence are of special interest. Recent studies presented autoantibodies targeting the adrenergic β_2 -receptor (β_2 -AR) in sera of patients with open-angle glaucoma. These β_2 -autoantibodies activate their target (β_2 -agAAb) with consecutive overstimulation and desensitization of the receptor contrary to physiological agents. *In vivo* data suggested a link of the β_2 -agAAb to the increased IOP and impaired retinal microcirculation. (Junemann et al., 2018; Hohberger et al., 2021a) We propose that a chronic overstimulation of the β_2 -AR can result in a dysregulated homeostatic adrenergic balance. Receptor desensitization and internalization might be only one of the molecular features (Wallukat, 2002). If this adrenergic disbalance has sufficient 'power' on cellular level, clinical characteristics occur in the patients (*imbalanced autonomic theory*; see also (Szentiványi, 1968)). This adrenergic disbalance is certainly not only a local problem (in the eye). We assume that adrenergic alterations might be even present in extraocular tissue of neurodegenerative diseases. Since several years a common pathomechanism of glaucoma and Alzheimer's Disease (AD) has been suggested. (Mancino et al., 2018; Inyushin et al., 2019; Rawlyk and Chauhan, 2021) Patients with AD show amyloid beta (A β) depositions being proposed to be the major mediator of neurotoxicity in the brain. (Wyss-Coray, 2006) A β is assumed to be one crosslink between glaucoma and AD. RGC express A β (den Haan et al., 2018; Lee et al., 2020). Especially, intravitreal injection of A β -42 was observed to induce RGC loss (Simons et al., 2021). A β is generated after cleavage of the amyloid precursor protein (APP). Ni et al. has shown that a β_2 -adrenergic stimulation that activates the gamma secretase is involved in the formation of A β from the APP (Ni et al., 2006). This cleavage can be mediated by vessel endothelial cell enzymes and platelets, especially when platelets contact the endothelium. Any thrombosis can enhance this A β generation. Functional AAb against the β_2 -AR were not only seen in the sera of patients with glaucoma but also in sera of patients with AD symptoms (Wallukat et al., 2018). These β_2 -agAAb but also the long change A β peptides prevent the desensitization of the β_2 AR and induce a permanent stimulation of this adrenergic receptor. As β_2 -agAAb show a link to an impaired microcirculation (glaucoma) and A β expression is increased during impaired microcirculation (i.e. during and after thrombosis; AD), we hypothesize that there is a common molecular mechanisms between adrenergic dysfunction (β_2 -agAAb; (I)) and A β (II). The aim of the study was to investigate sera of patients with AD (I) for the

presence of autoantibodies against β 2-AR, and (II) the effect of fragments and the complete A β peptides on the β 2-AR and whose desensitization.

Material and Methods

Patients and controls

Sera of 11 subjects with AD (3 female, 8 male, age: 40-87 years) and 18 subjects with primary open-angle glaucoma (7 female, 11 male, age: 42-76 years) were collected for the present study. Glaucoma patients were recruited at the Department of Ophthalmology and Eye Hospital, Friedrich-Alexander-University Erlangen-Nürnberg [Erlangen Glaucoma Registry, ISSN 2191-5008, CS-2011; NTC00494923]. (Hohberger et al., 2019) Patients with AD were recruited at the Department of Neurology, Charité-Universitätsmedizin Berlin. Blood samples were collected, centrifuged and sera were stored at -20°C. All individuals declared informed consent. The study was performed according to the tenets of the Declaration of Helsinki and approved by the ethic committee of the university of Erlangen, the institutional Review Board of Charité-Universitätsmedizin Berlin and the Max-Delbrück-Centrum for Molecular Medicine, Berlin-Buch (Y 9004/19, 02.11.2020).

Primary Open-Angle Glaucoma (POAG) Patients

The criteria of POAG were: an open anterior chamber angle, IOP>21 mmHg (confirmed at least once, measured by Goldmann applanation tonometry), and a glaucomatous optic disc, classified after Jonas. (Jonas et al., 1988) Functional loss of perimetric field were measured and had to be confirmed at least once according to the following criteria: (I) at least three adjacent test points having a deviation ≥ 5 dB and with one test point with a deviation >10 dB lower than normal, or (II) at least two adjacent test points with a deviation ≥ 10 dB, or (III) at least three adjacent test points with a deviation ≥ 5 dB abutting the nasal horizontal meridian or (IV) a mean visual field defect of >2.6 dB.

Cardiomyocyte bioassay

The cardiomyocyte bioassay was done as described previously (Junemann et al., 2018). Cell culture of cardiac myocytes of heart ventricle (1-3 day-old Sprague-Dawley rats) was used. digested with a 0.25% solution of crude porcine trypsin (Serva, Germany). The cells were dispersed by trypsinization and suspended in a SM20-I medium (Biochrom, Germany), glutamine (Serva, Germany), containing streptomycin (HEFA Pharma; Germany), penicillin (Heyl, Germany), hydrocortisone (Merck, Germany), fluorodeoxyuridine (Serva, Germany), and 10% heat-inactivated neonatal calf serum (Gibco, Germany). The cells were seeded (field density of 160.000 cells/cm²) the culture medium was replaced after 24 hours. The cardiomyocytes were cultured for 3-4 days (37°C) before the used in the experiments. Firstly, the basal beating rate of the cardiomyocytes was measured and subsequently the immunoglobulin (Ig) fractions were added for 60 min in a dilution of 1:40. Next, the beating rates (BR) of the cardiomyocytes were counted again on 6 marked spontaneously beating cells or cell cluster (for 15 seconds; at a heated stage of an inverted microscope at 37°C). 1 Unit reflects 4 beats/min (i.e. adrenergic activity). Samples below 2 Units of adrenergic activity were considered as normal. Application of a specific β 2-blocker, i.e. ICI 118.551 (0.1 μ M) was done for identification of the

receptor type.

Preparation of the serum Immunoglobulins

Human IgG were prepared from each patients' sera by direct ammonium sulfate precipitation (final concentration of 40%; overnight at 4°C). The precipitates were centrifuged (4,000 × g; 30 min) and the pellets were dissolved in dialysis buffer (154 mmol/l NaCl, 10 mmol/l Na₂HPO₄/NaH₂PO₄, pH 7.2). This procedure was repeated twice. Each sample was dialyzed against phosphate-buffered saline (4°C; 3 days). Storage of the samples was done at -20°C.

Aβ peptides

The fragmented Aβ peptides were synthesized by Dr. Beyermann (FMP Berlin). The long change Aβ peptides Aβ 1-40, Aβ 1-42 and also Aβ (Pyr) 3-42 were from Sigma-Aldrich.

Statistical analysis

Statistical analysis was done by SPSS (version 21) and Excel. Data are shown as absolute value, mean and standard deviation.

Results

Patients with glaucoma and Alzheimer's Disease

Patients with POAG showed a mean beat rate of 4.5±0.1 U (range: 3.3-5.2 U) that was realized via the β₂-AR. This increase in the beat rate could be blocked by the specific β₂-blocker ICI 118.551 (Figure 1). These fAAB recognize the second extracellular loop of the β₂-AR (Junemann et al., 2018). Functional AAB against the β₂-adrenoceptor prepared from patients with AD showed a mean beating rate of 4.5±0.4 U (range: 2.3-7.2 U). As observed for patients with POAG, this increase in the beat rate could be blocked by the specific β₂-blocker ICI 118.551 (Figure 1). Thus, a seropositivity for β₂-AAB was observed for patients with POAG and AD. However, the β₂-AR fAAB prepared from AD patient sera recognize the first extracellular loop of the β₂-AR (Wallukat et al., 2018). Moreover, the activation with the β₂-AAB induced in contrast to the classical β₂-AR agonists a permanent stimulation of the β₂-AR mediated signal cascade without desensitization (Figure 2).

In vitro analysis of amyloid β (Aβ) peptides effects

In order to identify a possible functional action of Aβ peptides on the GPCR different species of amyloid beta (Aβ) peptide were tested in the used bioassay. Following Aβ peptides were investigated: Aβ1-14, Aβ10-37, Aβ 25-35, Aβ 28-40, Aβ1-40, Aβ1-42, and [Pyr]-Aβ3-43). Our results demonstrate that only the Aβ peptide [Pyr] -Aβ 3-43, but not the other Aβ peptides or Aβ fragments are able to activate the β₂ adrenoceptor similar as the classical β-adrenergic agonists. The long-chain [Pyr]-Aβ3-42, representing a major neurogenic plaque component, exerted an activation of the β₂-AR blocked by the β₂-AR antagonist ICI 118.551 but not by the β₁-AR antagonists bisoprolol or the α₁-AR blocker urapidil (Figure 3). Moreover, the long chain Aβ1-40, Aβ 1-42, and Aβ 10-37 but not the short-chain Aβ-peptides prevented like the fAAB directed against the β₂-AR the clenbuterol induced desensitization of the β₂-AR (Figure 4, table 1).

Discussion

Glaucoma disease is one of the leading causes of irreversible blindness in the developing countries. Its complex pathogenesis is known to be multifactorial with an immune and autoimmune involvement. Clinical studies observed that this neurodegenerative ocular disorder shows several common features with neurodegenerative disorders of the brain (e.g. AD). (Criscuolo et al., 2017; Ramirez et al., 2017; Mancino et al., 2018; Rawlyk and Chauhan, 2021) Thus, it is commonly accepted that both disease entities seem to have common molecular pathways and clinical characteristics. To the best of our knowledge the present study shows a common pathogenic involvement of the β 2-adrenergic pathway both in AD and glaucoma for the first time. In sera of patients with AD and glaucoma a seropositivity for β 2-agAAb was observed, respectively. In addition, the long-chain [Pyr]-A β 3-42, representing the major neurogenic plaque component, showed a direct activation of the β 2-AR that was blocked by the specific antagonist IC118.551. This N-terminal truncated isoform of A β can trigger neurodegeneration in a transgenic mouse model (Wirths, 2009) and developed a high neurotoxicity (Tekirian, 1999). The other functional active A β peptides realize their effect in the used test model not via the β 2 adrenoceptor. Another aspect of the effects of the A β peptides exist in fact that the long chain A β 1-40, A β 1-42, A β 28-40 peptides prevented the clenbuterol induced desensitization of the β 2-AR similar as the β 2-adrenoceptor autoantibodies. All three mechanisms result finally in a boosted activation and permanent stimulation of the β 2-adrenergic pathways, potentially causing a dangerous adrenergic overdrive realized via the β 2-AR.

Glaucoma and AD disease were observed to share common features clinically and biochemically. Patients with open-angle glaucoma are at higher risk (Lin et al., 2014) and showed a higher incidence of AD (Moon et al., 2018). Vis-a-vis patients with AD are at higher risk of glaucoma (Lai et al., 2017). Both, glaucoma and AD, were observed to be age-related disorders (Klein et al., 1995; Molinuevo and Casado-Naranjo, 2014; Doucette et al., 2015; Guerreiro and Bras, 2015; McMonnies, 2017). As the eye, especially retinal tissue, represents “cerebral tissue” considering embryology, it is not remarkable that neurodegeneration and amyloid- β plaques were seen within the retina in AD patients (Whitehouse et al., 1981; Hinton et al., 1986; van de Nes et al., 2008). Degeneration of retinal ganglion cells (RGC) locally and retrograde degeneration of axons in the lateral geniculate body are two findings, being present in AD patients (Hinton et al., 1986; Sadun and Bassi, 1990; Braak and Braak, 1997; Simons et al., 2021). Even amyloid- β can induce RGC loss within the retina itself (Simons et al., 2021). These neurodegenerative features were also observed in glaucomatous eyes (Lee et al., 2014; Russo et al., 2016; Schmidt et al., 2018; Kosior-Jarecka et al., 2020). The diagnostic correlate of RGC loss can be seen in retinal nerve fiber layer thinning (RNFL) *in vivo*, visualized by magnet resonance tomography and optical coherence tomography (OCT) (Schmidt et al., 2018; Kosior-Jarecka et al., 2020). Both, patients with glaucoma and AD, show a RNFL thinning especially in the superior and/or inferior retina (Blanks et al., 1996a; Blanks et al., 1996b; Sehi et al., 2012; Schrems et al., 2017). Thus, it can be hypothesized that there is common pathway, linking both neurodegenerative disorders (glaucoma and AD).

The autonomic system is a main actor in human body. It regulates important function and is the basis of the human live. Any alterations in the sympathetic and/or parasympathetic pathways results in an impairment or overaction of the autonomic system. Adrenergic receptors are also present on pericytes, thus it can be hypothesized that any alterations in adrenergic activity might influence

microcirculation. It is commonly accepted that an impaired microcirculation can induce amyloid plaques in AD (Ishimaru et al., 1996; Armstrong, 2006; Zhang et al., 2020) and is a very early pathogenetic alteration in glaucoma disease (Grieshaber et al., 2007; Wey et al., 2019; Hohberger et al., 2021b). Up to now, it is still elusive which factors induce this impaired microcirculation in AD. Interestingly, recent data showed that an impaired microcirculation is able to change the amyloid- β plaques in a mouse model (Zhang et al., 2020). Even in glaucoma pathogenesis, the exact pathogenesis is under investigation. There is evidence that an involvement of the autonomic system, especially the adrenergic β 2-mediated pathway might be present in dysregulation of IOP (glaucoma) (Hohberger et al., 2020; Hohberger et al., 2021c) and capillary microcirculation (glaucoma (Hohberger et al., 2021a) and AD), as patients with glaucoma and AD showed a seropositivity for β 2-AAb, respectively.

It was shown that patients with AD develop functional active autoantibodies directed against the β 2-AR. These β 2-agAAb activate the β 2-AR in a similar manner as the β 2 adrenergic agonists (Wallukat et al., 2018) but in contrast to the agonists the β 2-agAAb prevent the desensitization of the corresponding receptor. This observation was also made in glaucoma patients (Junemann et al., 2018). The agonist-like effect of this β 2-agAAb was blocked by ICI118.551, yet not by the β -AR antagonist bisoprolol. Moreover, the agonistic effect of the β 2-agAAb was neutralized by a peptide corresponding to the second extracellular loop of this receptor. In contrast to the classical β -AR agonists, β 2-agAAb activate the β 2-AR mediated signal cascade permanently. Moreover, the β 2-agAAb also prevent the desensitization, normally seen for the classical β 2-AR agonists, when agonists were added to β 2-agAAb pre-stimulated cardiomyocytes. This permanent stimulation without desensitization of the β 2-AR may represent a pathogenic factor that played an essential role in the pathogenesis of several diseases (Wallukat et al., 1991). Another feature of these β 2-agAAb is the possible antagonistic action of these β 2-agAAb directed against the β 2-AR. It was shown by Mijares et al. that monovalent Fab fragments of monoclonal β 2-agAbs exhibit antagonistic and not agonistic properties (Mijares et al., 2000). However, if these monovalent Fab fragments were crosslinked by an anti Fab antibody than the agonist-like effects were restored (Wallukat and Schimke, 2014). These data demonstrate that the β 2-agAAb also can act in an antagonistic manner under particular circumstances.

In patients with AD disease the β 2-adrenergic dysregulation might be mediated in three different ways. First is the direct activation of the β 2-AR by its classical natural agonist adrenaline or synthetic compound like isoprenaline. Both compounds activate the β 1-, the β 2- and the β 3-AR. The stimulation with adrenaline, isoprenaline or the more β 2-AR specific agonist clenbuterol induced a positive chronotropic response in the cardiomyocyte bioassay, which could be blocked by the specific β 2-AR antagonist ICI118.551. Furthermore, clenbuterol caused like the most agonists during a permanent treatment a desensitization of the β 2-AR mediated response. After an incubation time of two hours, a washing procedure and a new stimulation with the same agonist concentration the cardiomyocytes respond only with a third of maximal response (Fig. 2a).

Secondly, it was shown that the activation of the β 2-AR may play a role in the formation of amyloid β (A β). β 2-AR agonists activate the gamma-secretase, forming A β from the amyloid precursor proteins (APP), resulting in an elevated accumulation of A β (Ni et al., 2006). Moreover, a micro perfusion of A β 1-40 in combination with the β 2-adrenergic agonist clenbuterol into the hippocampus caused an augmented degradation of the A β 1:40 peptide in comparison to the controls without clenbuterol

(Richter R.M, 2010). Some of these degradation products of the A β 1-40 and A β 1-40 itself can stimulate different G-Protein coupled receptors (GPCR) in a similar manner as the classical receptor agonists. For example, the A β -fragments A β 25-35 and A β 10-37 activate the α 1-AR and this activation was abolished by the antagonist prazosin or urapidil. These A β -peptides recognize the first extracellular loop of the α 1-adrenergic receptor and the A β 25-35 and A β 10-37 induced activation could be blocked by peptides corresponding to the first extracellular loop (Haase et al., 2013). Such α 1-adrenoceptor antibodies can influence moderately the contractility of the coronary and middle cerebral artery and strongly the renal artery of the rat (Yan et al., 2009). Moreover, Tohda et al. demonstrated that A β 25-35 induced memory impairment, axonal atrophy and synaptic loss (Tohda et al., 2004).

Wang and coworker (a,b) observed in prefrontal cortical neurons that A β 1-42 can bind to the β 2-AR, and activate the protein kinase A (PKA) in these neurons (Wang et al., 2010; Wang et al., 2011). This observation was not confirmed in our experiments using a functional cardiomyocyte assay. In our bioassay A β 1-40, A β 1-42 but also A β 1-14 activate the fast estradiol receptor GPR 30 (unpublished data). In contrast, the N-terminal truncated A β peptide [Pyr] 3-43 that representing a major neuritic plaque component was able to activate the β 2-AR subtype in our cardiomyocyte model. This A β peptide, activate the β 2-AR in similar manner as the agonist clenbuterol. This activation was neither influenced by the α 1-adrenergic antagonist urapidil nor the β 1-antagonist bisoprolol. Only ICI118.551 was able to block the activity induced by A β [Pyr] 3-43. Therefore, we assume that in the experiments of Wang on neuronal tissues the A β -peptides A β 1-40 and A β 1-42 were truncated to A β [Pyr] 3-43 by endogenous peptidases that can recognize the β 2-AR subtype.

A third possibility to influence the β 2-AR activity can be seen in fact that the long chain A β peptides A β 1-42, A β 1-40, and A β 10-37, yet not the short breaking products of the amyloid A β 1-14, A β 25-35 and A β 28-40 prevent desensitization of the β 2-adrenergic agonist clenbuterol. The combination of the long chain A β peptides and clenbuterol induced a permanent activation of the β 2-signal cascade similar to the response seen for the agonist-like β 2-AAbs. This observation indicates that the long chain A β can also contribute on the adrenergic overdrive.

We hypothesize that a dysregulated β 2-mediated signal cascade might be a molecular mechanism of an autonomic dysregulation, both observed in neurodegenerative disorders, e.g. glaucoma and AD. A seropositivity for β 2-AAb and the β -adrenergic agonists itself can enhance β 2-AR activation. Additionally, to β 2-AAb and the A β peptide A β [Pyr] 3-43 were also able to activate this signal cascade directly. Furthermore, the long chain A β peptides induce like the β 2-AAb a permanent stimulation and deactivate the protection mechanism of the cells against an adrenergic overstimulation. All these mechanisms may play an important role in the pathogenesis of Glaucoma and AD.

Conclusion

Glaucoma and AD disorder are known to be neurodegenerative disorders with an involvement of autoimmune mechanisms and impaired microcirculation. As both disease entities showed a seropositivity for β 2-AAb we postulate that an overstimulated β 2-AR pathway can induce an adrenergic overdrive, which can be enhanced by amyloid- β peptides in AD patients.

References

- Armstrong, R.A. (2006). Plaques and tangles and the pathogenesis of Alzheimer's disease. *Folia Neuropathol* 44, 1-11.
- Blanks, J.C., Schmidt, S.Y., Torigoe, Y., Porrello, K.V., Hinton, D.R., and Blanks, R.H. (1996a). Retinal pathology in Alzheimer's disease. II. Regional neuron loss and glial changes in GCL. *Neurobiol Aging* 17, 385-395.
- Blanks, J.C., Torigoe, Y., Hinton, D.R., and Blanks, R.H. (1996b). Retinal pathology in Alzheimer's disease. I. Ganglion cell loss in foveal/parafoveal retina. *Neurobiol Aging* 17, 377-384.
- Braak, H., and Braak, E. (1997). Staging of Alzheimer-related cortical destruction. *Int Psychogeriatr* 9 Suppl 1, 257-261; discussion 269-272.
- Chen, H., Cho, K.S., Vu, T.H.K., Shen, C.H., Kaur, M., Chen, G., Mathew, R., Mcham, M.L., Fazelat, A., Lashkari, K., Au, N.P.B., Tse, J.K.Y., Li, Y., Yu, H., Yang, L., Stein-Streilein, J., Ma, C.H.E., Woolf, C.J., Whary, M.T., Jager, M.J., Fox, J.G., Chen, J., and Chen, D.F. (2018). Commensal microflora-induced T cell responses mediate progressive neurodegeneration in glaucoma. *Nat Commun* 9, 3209.
- Criscuolo, C., Fabiani, C., Cerri, E., and Domenici, L. (2017). Synaptic Dysfunction in Alzheimer's Disease and Glaucoma: From Common Degenerative Mechanisms Toward Neuroprotection. *Front Cell Neurosci* 11, 53.
- Den Haan, J., Morrema, T.H.J., Verbraak, F.D., De Boer, J.F., Scheltens, P., Rozemuller, A.J., Bergen, A.a.B., Bouwman, F.H., and Hoozemans, J.J. (2018). Amyloid-beta and phosphorylated tau in post-mortem Alzheimer's disease retinas. *Acta Neuropathol Commun* 6, 147.
- Doucette, L.P., Rasnitsyn, A., Seifi, M., and Walter, M.A. (2015). The interactions of genes, age, and environment in glaucoma pathogenesis. *Surv Ophthalmol* 60, 310-326.
- Gramlich, O.W., Godwin, C.R., Heuss, N.D., Gregerson, D.S., and Kuehn, M.H. (2020). T and B Lymphocyte Deficiency in Rag1^{-/-} Mice Reduces Retinal Ganglion Cell Loss in Experimental Glaucoma. *Invest Ophthalmol Vis Sci* 61, 18.
- Grieshaber, M.C., Mozaffarieh, M., and Flammer, J. (2007). What is the link between vascular dysregulation and glaucoma? *Surv Ophthalmol* 52 Suppl 2, S144-154.
- Guerreiro, R., and Bras, J. (2015). The age factor in Alzheimer's disease. *Genome Med* 7, 106.
- Haase, N., Herse, F., Spallek, B., Haase, H., Morano, I., Qadri, F., Szijarto, I.A., Rohm, I., Yilmaz, A., Warrington, J.P., Ryan, M.J., Gollasch, M., Muller, D.N., Dechend, R., and Wallukat, G. (2013). Amyloid-beta peptides activate alpha1-adrenergic cardiovascular receptors. *Hypertension* 62, 966-972.
- Hinton, D.R., Sadun, A.A., Blanks, J.C., and Miller, C.A. (1986). Optic-nerve degeneration in Alzheimer's disease. *N Engl J Med* 315, 485-487.
- Hohberger, B., Hosari, S., Wallukat, G., Kunze, R., Krebs, J., Muller, M., Hennig, T., Lammer, R., Horn, F., Munoz, L.E., Herrmann, M., and Mardin, C. (2021a). Agonistic autoantibodies against beta2-adrenergic receptor influence retinal microcirculation in glaucoma suspects and patients. *PLoS One* 16, e0249202.
- Hohberger, B., Lucio, M., Schlick, S., Wollborn, A., Hosari, S., and Mardin, C. (2021b). OCT-angiography: Regional reduced macula microcirculation in ocular hypertensive and pre-perimetric glaucoma patients. *PLoS One* 16, e0246469.

- Hohberger, B., Monczak, E., and Mardin, C.Y. (2019). [26 Years of the Erlangen Glaucoma Registry: Demographic and Perimetric Characteristics of Patients Through the Ages]. *Klin Monbl Augenheilkd* 236, 691-698.
- Hohberger, B., Worn, M., Lammer, R., Herrmann, M., Mardin, C.Y., Kruse, F.E., Kunze, R., and Wallukat, G. (2020). Agonistic beta 2-adrenergic receptor autoantibodies characterize the aqueous humor of patients with primary and secondary open-angle glaucoma. *Investigative Ophthalmology & Visual Science* 61.
- Hohberger, B., Worn, M., Lammer, R., Mahajan, A., Mardin, C., Schotzer-Schrehardt, U., Kunze, R., Herrmann, M., and Wallukat, G. (2021c). Agonistic beta2-Adrenergic Receptor Autoantibodies Characterize the Aqueous Humor of Patients With Primary and Secondary Open-Angle Glaucoma. *Front Immunol* 12, 550236.
- Inyushin, M., Zayas-Santiago, A., Rojas, L., Kucheryavykh, Y., and Kucheryavykh, L. (2019). Platelet-generated amyloid beta peptides in Alzheimer's disease and glaucoma. *Histology and Histopathology* 34, 843-856.
- Ishimaru, H., Ishikawa, K., Haga, S., Shoji, M., Ohe, Y., Haga, C., Sasaki, A., Takashashi, A., and Maruyama, Y. (1996). Accumulation of apolipoprotein E and beta-amyloid-like protein in a trace of the hippocampal CA1 pyramidal cell layer after ischaemic delayed neuronal death. *Neuroreport* 7, 3063-3067.
- Jonas, J.B., Gusek, G.C., and Naumann, G.O. (1988). Optic disc morphometry in chronic primary open-angle glaucoma. I. Morphometric intrapapillary characteristics. *Graefes Arch Clin Exp Ophthalmol* 226, 522-530.
- Junemann, A., Hohberger, B., Rech, J., Sheriff, A., Fu, Q., Schlotzer-Schrehardt, U., Voll, R.E., Bartel, S., Kalbacher, H., Hoebeke, J., Rejdak, R., Horn, F., Wallukat, G., Kunze, R., and Herrmann, M. (2018). Agonistic Autoantibodies to the beta2-Adrenergic Receptor Involved in the Pathogenesis of Open-Angle Glaucoma. *Front Immunol* 9, 145.
- Klein, R., Klein, B.E., and Moss, S.E. (1995). Age-related eye disease and survival. The Beaver Dam Eye Study. *Arch Ophthalmol* 113, 333-339.
- Kosior-Jarecka, E., Pankowska, A., Polit, P., Stepniewski, A., Symms, M.R., Koziol, P., Zarnowski, T., and Pietura, R. (2020). Volume of Lateral Geniculate Nucleus in Patients with Glaucoma in 7Tesla MRI. *J Clin Med* 9.
- Lai, S.W., Lin, C.L., and Liao, K.F. (2017). Glaucoma may be a non-memory manifestation of Alzheimer's disease in older people. *Int Psychogeriatr*, 1-7.
- Lee, J.Y., Jeong, H.J., Lee, J.H., Kim, Y.J., Kim, E.Y., Kim, Y.Y., Ryu, T., Cho, Z.H., and Kim, Y.B. (2014). An investigation of lateral geniculate nucleus volume in patients with primary open-angle glaucoma using 7 tesla magnetic resonance imaging. *Invest Ophthalmol Vis Sci* 55, 3468-3476.
- Lee, S., Jiang, K., Mcilmoyle, B., To, E., Xu, Q.A., Hirsch-Reinshagen, V., Mackenzie, I.R., Hsiung, G.R., Eadie, B.D., Sarunic, M.V., Beg, M.F., Cui, J.Z., and Matsubara, J.A. (2020). Amyloid Beta Immunoreactivity in the Retinal Ganglion Cell Layer of the Alzheimer's Eye. *Front Neurosci* 14, 758.
- Lin, I.C., Wang, Y.H., Wang, T.J., Wang, I.J., Shen, Y.D., Chi, N.F., and Chien, L.N. (2014). Glaucoma, Alzheimer's disease, and Parkinson's disease: an 8-year population-based follow-up study. *PLoS One* 9, e108938.
- Mancino, R., Martucci, A., Cesareo, M., Giannini, C., Corasaniti, M.T., Bagetta, G., and Nucci, C. (2018). Glaucoma and Alzheimer Disease: One Age-Related Neurodegenerative Disease of the Brain. *Curr Neuroparmacol* 16, 971-977.
- Mcmonnies, C.W. (2017). Glaucoma history and risk factors. *J Optom* 10, 71-78.
- Mijares, A., Lebesgue, D., Wallukat, G., and Hoebeke, J. (2000). From agonist to antagonist: Fab fragments of an agonist-like monoclonal anti-beta(2)-adrenoceptor antibody behave as antagonists. *Mol Pharmacol* 58, 373-379.
- Molinuevo, J.L., and Casado-Naranjo, I. (2014). Clinical profile of Alzheimer's disease: is the age of the patient a decisive factor? Results of the INFLUENCE study. *J Alzheimers Dis* 39, 227-232.

- Moon, J.Y., Kim, H.J., Park, Y.H., Park, T.K., Park, E.C., Kim, C.Y., and Lee, S.H. (2018). Association between Open-Angle Glaucoma and the Risks of Alzheimer's and Parkinson's Diseases in South Korea: A 10-year Nationwide Cohort Study. *Sci Rep* 8, 11161.
- Ni, Y., Zhao, X., Bao, G., Zou, L., Teng, L., Wang, Z., Song, M., Xiong, J., Bai, Y., and Pei, G. (2006). Activation of beta2-adrenergic receptor stimulates gamma-secretase activity and accelerates amyloid plaque formation. *Nat Med* 12, 1390-1396.
- Ramirez, A.I., De Hoz, R., Salobrar-Garcia, E., Salazar, J.J., Rojas, B., Ajoy, D., Lopez-Cuenca, I., Rojas, P., Trivino, A., and Ramirez, J.M. (2017). The Role of Microglia in Retinal Neurodegeneration: Alzheimer's Disease, Parkinson, and Glaucoma. *Front Aging Neurosci* 9, 214.
- Rawlyk, B., and Chauhan, B.C. (2021). Retinal ganglion cell loss in postmortem tissue of Alzheimer disease, glaucoma, and healthy normal subjects. *Can J Ophthalmol* 56, e66-e70.
- Richter R.M, B.M., Schulze W., Wallukat G. (2010). "Impact of β 2- activation on the processing of amyloid- β peptides in the rat hippocampus", in: *Neuroscience Meeting Planner (San Diego)*).
- Russo, R., Varano, G.P., Adornetto, A., Nucci, C., Corasaniti, M.T., Bagetta, G., and Morrone, L.A. (2016). Retinal ganglion cell death in glaucoma: Exploring the role of neuroinflammation. *Eur J Pharmacol* 787, 134-142.
- Sadun, A.A., and Bassi, C.J. (1990). Optic nerve damage in Alzheimer's disease. *Ophthalmology* 97, 9-17.
- Schmidt, M.A., Knott, M., Heidemann, R., Michelson, G., Kober, T., Dorfler, A., and Engelhorn, T. (2018). Investigation of lateral geniculate nucleus volume and diffusion tensor imaging in patients with normal tension glaucoma using 7 tesla magnetic resonance imaging. *PLoS One* 13, e0198830.
- Schrems, W.A., Schrems-Hoesl, L.M., Mardin, C.Y., Laemmer, R., Kruse, F.E., and Horn, F.K. (2017). Can Glaucomatous Visual Field Progression be Predicted by Structural and Functional Measures? *J Glaucoma* 26, 373-382.
- Sehi, M., Bhardwaj, N., Chung, Y.S., Greenfield, D.S., and Advanced Imaging for Glaucoma Study, G. (2012). Evaluation of baseline structural factors for predicting glaucomatous visual-field progression using optical coherence tomography, scanning laser polarimetry and confocal scanning laser ophthalmoscopy. *Eye (Lond)* 26, 1527-1535.
- Simons, E.S., Smith, M.A., Dengler-Crish, C.M., and Crish, S.D. (2021). Retinal ganglion cell loss and gliosis in the retinofugal projection following intravitreal exposure to amyloid-beta. *Neurobiol Dis* 147, 105146.
- Szentiványi, A. (1968). The beta-adrenergic theory of the atopic abnormality in bronchial asthma. *J Allergy* 42, 203-232.
- Tohda, C., Matsumoto, N., Zou, K., Meselhy, M.R., and Komatsu, K. (2004). A beta(25-35)-induced memory impairment, axonal atrophy, and synaptic loss are ameliorated by M1, a metabolite of protopanaxadiol-type saponins. *Neuropsychopharmacology* 29, 860-868.
- Van De Nes, J.A., Nafe, R., and Schlote, W. (2008). Non-tau based neuronal degeneration in Alzheimer's disease -- an immunocytochemical and quantitative study in the supragranular layers of the middle temporal neocortex. *Brain Res* 1213, 152-165.
- Wallukat, G. (2002). The beta-adrenergic receptors. *Herz* 27, 683-690.
- Wallukat, G., Morwinski, M., Kowal, K., Forster, A., Boewer, V., and Wollenberger, A. (1991). Autoantibodies against the beta-adrenergic receptor in human myocarditis and dilated cardiomyopathy: beta-adrenergic agonism without desensitization. *Eur Heart J* 12 Suppl D, 178-181.
- Wallukat, G., Pruss, H., Muller, J., and Schimke, I. (2018). Functional autoantibodies in patients with different forms of dementia. *PLoS One* 13, e0192778.
- Wallukat, G., and Schimke, I. (2014). Agonistic autoantibodies directed against G-protein-coupled receptors and their relationship to cardiovascular diseases. *Semin Immunopathol* 36, 351-363.

- Wang, D., Govindaiah, G., Liu, R., De Arcangelis, V., Cox, C.L., and Xiang, Y.K. (2010). Binding of amyloid beta peptide to beta2 adrenergic receptor induces PKA-dependent AMPA receptor hyperactivity. *FASEB J* 24, 3511-3521.
- Wang, D., Yuen, E.Y., Zhou, Y., Yan, Z., and Xiang, Y.K. (2011). Amyloid beta peptide-(1-42) induces internalization and degradation of beta2 adrenergic receptors in prefrontal cortical neurons. *J Biol Chem* 286, 31852-31863.
- Wey, S., Amanullah, S., Spaeth, G.L., Ustaoglu, M., Rahmatnejad, K., and Katz, L.J. (2019). Is primary open-angle glaucoma an ocular manifestation of systemic disease? *Graefes Arch Clin Exp Ophthalmol* 257, 665-673.
- Whitehouse, P.J., Price, D.L., Clark, A.W., Coyle, J.T., and Delong, M.R. (1981). Alzheimer disease: evidence for selective loss of cholinergic neurons in the nucleus basalis. *Ann Neurol* 10, 122-126.
- Wyss-Coray, T. (2006). Inflammation in Alzheimer disease: driving force, bystander or beneficial response? *Nat Med* 12, 1005-1015.
- Yan, L., Xu, Y., Yao, H., Xue, W., Tian, J., Ren, H., Wu, Y., Yang, G., Ma, X.L., and Liu, H. (2009). The effects of autoantibodies against the second extracellular loop of alpha(1)-adrenoceptor on vasoconstriction. *Basic Res Cardiol* 104, 581-589.
- Zhang, Y., Bander, E.D., Lee, Y., Muoser, C., Schaffer, C.B., and Nishimura, N. (2020). Microvessel occlusions alter amyloid-beta plaque morphology in a mouse model of Alzheimer's disease. *J Cereb Blood Flow Metab* 40, 2115-2131.

Legends:

- Fig. 1 Comparison of the activity of the functional β 2-adrenoceptor autoantibodies (β 2-AAb) of patients with glaucoma (POAG) and Alzheimer's disease (AD). Both agonist-like effects were blocked by the β 2-adrenoceptor antagonist ICI 118.551 (0.1 μ M). The experiments with the AAb of the AD patients were done in the presence of the α 1- adrenoceptor antagonist urapidil ($p < 0.05$).
- Fig.2 Desensitization of the β 2 adrenergic response by the β 2-adrenergic agonist clenbuterol (a). This receptor desensitization was missed if the cells were stimulated with the β 2-adrenergic AAb prepared from AD patients (b).
- Fig. 3 Functional effect realized via the β 2 adrenoceptor by the truncated A β peptide A β [PYR] 3-43: This effect was blocked by ICI 118.551, yet not by the β 1 adrenoceptor antagonist bisoprolol or the α 1-adrenergic antagonist urapidil.
- Fig. 4 Influence of the A β peptides on the clenbuterol induced β 2-adrenoceptor desensitization: the desensitization of the β -AR response by clenbuterol, was not influenced by the short chain A β peptides (a). However, the long chain A β peptides A β 10-37, A β 1-40, and A β 1-42 prevent the desensitization and exert a permanent stimulation of the β 2-AR signal cascade (b).

Tab.1 Influence of short and long chain Amyloid β peptides on the clenbuterol induced β 2-adrenoceptor desensitization.

Time		5'	60'	120'		125'		130'
Clenbuterol 1 μ M	Clenbuterol 1 μ M	10.2 $\pm$ 0.3	8.7 $\pm$ 0.3	5.9 $\pm$ 0.3	3 x wash	0.2 $\pm$ 0.1	Clenbuterol 1 μ M	2.7 $\pm$ 0.1
	+ A β 1-14	8.7 $\pm$ 0.6	6.7 $\pm$ 0.5	3.5 $\pm$ 0.5		0.2 $\pm$ 0.1		2.4 $\pm$ 0.1
	+ A β 25-35	8.9 $\pm$ 1.2	7.4 $\pm$ 1.0	5.7 $\pm$ 0.6		0.2 $\pm$ 0.1		2.9 $\pm$ 0.2
	+ A β 28-40	10.5 $\pm$ 0.2	7.8 $\pm$ 0.6	4.3 $\pm$ 0.6		0.3 $\pm$ 0.2		2.3 $\pm$ 0.2
	+ A β 1-40	10.8 $\pm$ 0.2	10.7 $\pm$ 0.4	9.8 $\pm$ 0.2		0.6 $\pm$ 0.1		9.3 $\pm$ 2.9
	+ A β 1-42	8.9 $\pm$ 0.1	9.3 $\pm$ 0.4	8.9 $\pm$ 0.4		0.2 $\pm$ 0.1		9.6 $\pm$ 0.3
	+ A β 10-37	9.6 $\pm$ 0.2	10.1 $\pm$ 0.3	8.2 $\pm$ 0.8		0.4 $\pm$ 0.1		10.3 $\pm$ 0.2

Increase in number of beats / 15 sec

POAG

AD

(a)

(b)

Increase in number of beats / 15 sec

(a)

Increase in number of beats / 15 sec

(b)

Increase in number of beats / 15 sec