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Immunohistochemical identification of kynurenine aminotransferases in corpora amylacea in the human retina and optic nerve

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Abstract

Introduction: *Corpora amylacea (CAm) occur in the optic nerve and in retinal ageing and degeneration. Cellular expression of L-kynurenine aminotransferases (KAT I and II) in the avian and rodent retina and its changes in retinal development and neurodegeneration have been documented. This study examines KAT I and II immunoreactivity in CAm in the human retina and optic nerve.*

Material and methods: *Immunohistochemistry was performed using polyclonal antibodies against KAT I and KAT II on sections of 23 human eyes enucleated for malignant uveal melanoma. Occurrence and location of KAT I- or KAT II-stained CAm was compared with PAS-stained sections.*

Results: *KAT I and KAT II expression in CAm has been shown in the retina and optic nerve with similar location to PAS-stained sections. KAT I immunoreactivity was more intense than KAT II and its staining was more pronounced in the retrolaminar part of the optic nerve. Some of the CAm showed only faint KAT II expression and occasionally there was no staining. KAT II revealed no association of staining variability and localisation of CAm. Similarly to animal studies, in the human retina KAT I was observed on Müller cell endfeet while KAT II was expressed in retinal ganglion cells.*

Conclusions: *Presence of kynurenine aminotransferases in CAm in the human retina and optic nerve suggest that both enzymes may be involved in mechanisms of retinal ageing and neurodegeneration leading to CAm formation.*

Key words: *corpora amylacea, L-kynurenine aminotranferases, degeneration, retina, optic nerve.*

Introduction

Corpora amylacea (CAm) are homogeneous or laminated oval structures frequently found in the brain and peripheral nerves. In the eye, CAm are observed in the optic nerve head, nerve fibre layer, ganglion cell layer, but also in the inner plexiform

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layer and inner nuclear layer [13,31]. Ultrastructurally, CAm consist of a mass of filamentous tangles within an axonal swelling [1,31]. Formation of CAm was suggested to result from impaired axonal flow [16].

In the central nervous system, CAm are regarded as a hallmark of ageing, and are thought to be associated with neurodegeneration [6,8,17], but little is known about their role in normal and pathological circumstances. Studies on the structure of CAm have shown that their rich acid polysaccharide content makes them best demonstrable by the PAS (periodic acid-Schiff) stain. CAm contain, in addition to glucose polymers, ageing, stress and proinflammatory proteins [4]. But, previous studies emphasised the surprising lack of their immunoreactivity by using many other antigens [14].

Importantly, data from various brain studies suggest that CAm possess a relatively high affinity to accumulate to some extent 'protective' substances which could rescue nerve cells from the devastating effects of ischaemia or ageing [4,6]. To test this hypothesis it was reasonable to extend studies on potential involvement of CAm in mechanisms of endogenous protection in the nervous system.

Kynurenine aminotransferases (KAT I and II) are pivotal to the synthesis of kynurenic acid (KYNA), the only known endogenous antagonist of glutamate [21], acetylcholine α7 nicotinic receptors [11] and neuroprotectant [29]. Presence of KATs and KYNA in the CNS and retina has been well documented [12,22-27, 30,33]. Investigations on KYNA are important because alterations of its synthesis are involved in the pathophysiology of several brain [3,18,32] and retinal disorders [22,23].

Therefore, to gain new insight into the role of CAm, this study is the first to examine the presence and patterns of KAT I and II immunoreactivity in CAm in the human retina and optic nerve.

Material and methods

Twenty-three human eyes from twenty-three patients [13 female, 10 male, age: 56-90] enucleated because of choroidal malignant melanoma were used for this study. The patients did not suffer from other ocular diseases. The study protocol was approved by the Human Ethics Committee of the University of Erlangen-Nuernberg.

All globes were fixed immediately after enucleation in a solution of 4% formaldehyde and 1% glutaraldehyde in 0.1% phosphate buffer (pH 7.2). Consecutive 5-µm sections including the centre of the disc and the pupil (P-O sections) were stained with PAS (periodic acid-Schiff), HE (hematoxilin-eosin) or subjected to immunohistochemistry.

Each of the eyes was stained with an anti-KAT I or anti-KAT II polyclonal antibody (1:50) [19,20] at least twice, using the streptavidin-biotin-method, as described previously [7]. Briefly, after deparaffinization and rehydration, sections were digested with proteinase K (Dako) before incubation with peroxidase for 10 minutes. Sections were then incubated with primary antibody (30 minutes) and horseradish peroxidase (HRP)-conjugated secondary antibody before development with 3-amino-9-ethylcarbazole (AEC)$^+$ substrate (red reaction product). Finally, the sections were counterstained with Mayer haemalaun (Chroma, Münster, Germany) and mounted in an aqueous-based medium (Faramount; Dako). Preimmune serum was included as the negative control and showed no staining of corpora amylacea. Sections were photographed with a microscope (Axiophot; Carl Zeiss, Oberkochen, Germany) using colour film (Ektachrome 64 T; Eastman Kodak, Rochester, NY).

Results

Light microscopically, all studied eyes revealed collateral retinal detachment and degeneration of the retina overlying the choroidal malignant melanoma. In PAS-stained sections CAm occurred as round, oval, smooth or laminated bodies with dense centres. CAm were observed in all cases in the optic nerve head and prelaminary, laminary and retrolaminary regions of the optic nerve (Fig. 1: R1, PL1, L1, RL1). In the retina, CAm were found in the inner plexiform layer, inner nuclear layer, ganglion cell layer and nerve fibre layer (data not shown). These findings are in agreement with previous results of Kubota and colleagues [13].

Immunohistochemistry showed the presence of both KAT I and KAT II immunoreactivity in CAm (Figs. 1, 2, 3, 4). KAT I immunoreactivity was observed in CAm in the retina (Fig. 1 R2) and prelaminary (Fig. 2 PL2), laminary (Fig. 3 L2) and retrolaminary (Fig. 4 RL2) regions of the optic nerve, and the pattern of its staining in most cases was intense. In general, there was more pronounced staining of KAT I in the retrolaminar part of the optic nerve (Fig. 4 RL2).

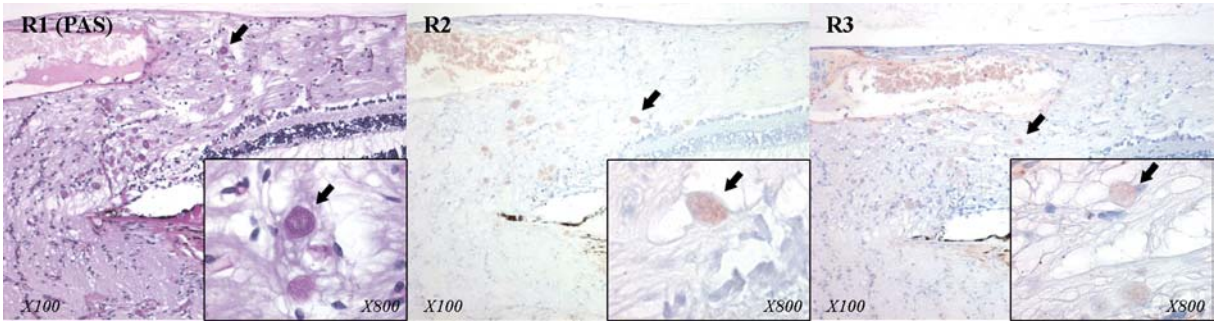

Fig. 1. PAS and immunohistochemical staining of KAT I and II (L-kynurenine aminotransferases I and II) in CAm in the human retina. In PAS-stained sections CAm [black arrows] were observed in all cases in the retina (R1, PAS). Immunoreactivity of KAT I was detected in CAm [black arrows] in the retina (R2) showing intense staining. KAT II staining was also observed in CAm [black arrows] in the retina (R3), being less pronounced than KAT I. Magnifications are indicated on the pictures

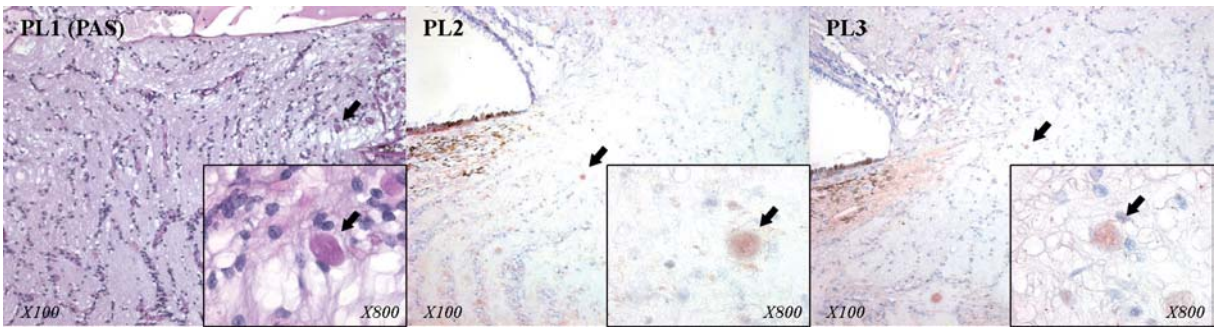

Fig. 2. PAS and immunohistochemical staining of KAT I and II (L-kynurenine aminotransferases I and II) in CAm in prelaminary region of the human optic nerve. In PAS-stained sections CAm [black arrows] were observed in all cases in the prelaminary (PL1, PAS) region of the optic nerve. KAT I immunoreactivity was observed in CAm [black arrows] in the prelaminary (PL2) region of the optic nerve and the pattern of its staining in most cases was intense. The presence of KAT II staining was found in CAm localised in preliminary (PL3) region of the optic nerve [black arrows]. Immunoreactivity of KAT II was less pronounced than KAT I. Magnifications are indicated on the pictures

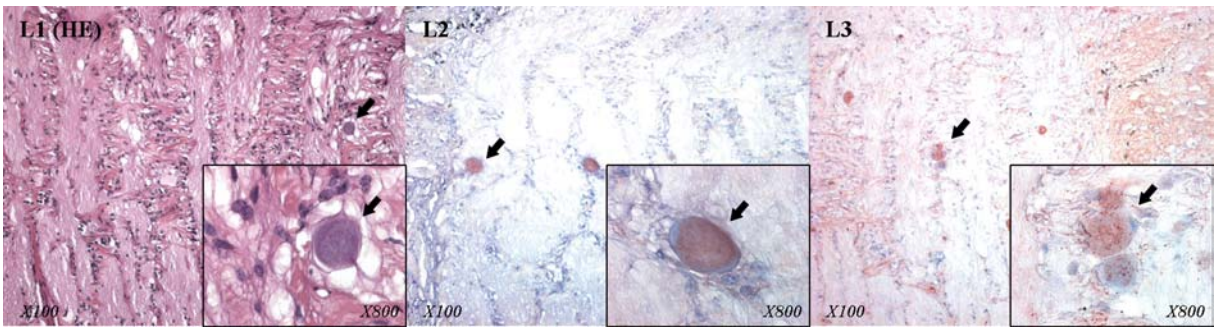

Fig. 3. HE and immunohistochemical staining of KAT I and II (L-kynurenine aminotransferases I and II) in CAm in laminary region of the human optic nerve. In HE-stained sections CAm were observed in all cases in the laminary (L1, HE) region of the optic nerve [black arrows]. KAT I immunoreactivity was observed in CAm [black arrows] in the laminary (L2) region of the optic nerve with intense pattern of its staining. KAT II expression was observed in CAm localised in the laminary region of the optic nerve (L3) [black arrows]. Immunoreactivity of KAT II was less pronounced than KAT I. Magnifications are indicated on the pictures

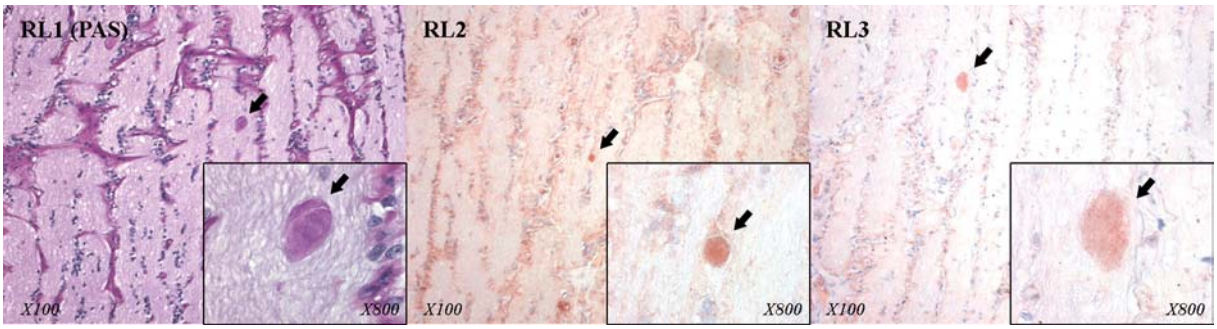

Fig. 4. PAS and immunohistochemical staining of KAT I and II (L-kynurenine aminotransferases I and II) in CAm in retrolaminary region of the human optic nerve. In PAS-stained sections CAm were observed in all cases in the retrolaminary (RL1, PAS) region of the optic nerve [black arrows]. KAT I immunoreactivity was observed in CAm [black arrows] in the retrolaminary (RL2) region of the optic nerve and the pattern of its staining in most cases was intense. In general, there was more pronounced staining in the retrolaminar part of the optic nerve as compared to the retina and other regions of the optic nerve. The presence of KAT II was observed in CAm [black arrows] localised in retrolaminary region of the optic nerve (RL3). Some CAm showed only faint KAT II staining (RL3) and occasionally there was no staining (pictures not shown). Immunoreactivity of KAT II was less pronounced than KAT I. There was no association of staining variety of KAT II and localisation of CAm. Magnifications are indicated on the pictures

The presence of KAT II expression was observed in CAm localised in both the retina and optic nerve (Fig. 1 R3; Fig. 2 PL3; Fig. 3 L3; Fig. 4 RL3). Some CAm showed only faint KAT II immunoreactivity and occasionally there was no staining (data not shown). Immunoreactivity of KAT II was less pronounced than KAT I. There was no association of staining variety of KAT II and localisation of CAm. Also, there was no correlation between size of CAm and immunoreactivity of both KAT I and KAT II.

Moreover, our studies revealed cellular expression of both isoforms of KAT in the human retina (Fig. 5). KAT I was preferentially localised on Müller cell endfeet while KAT II was expressed in retinal ganglion cells. These results parallel our previous observations in the rodent retina [22,23,26].

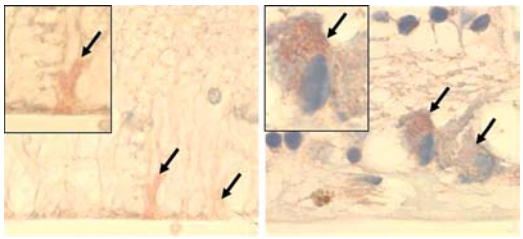

Fig. 5. Localisation of KAT I and KAT II immunoreactivity in radial sections of the human retina. The ganglion cell layer is oriented downward. (a) Labelling of KAT I. KAT I is expressed in Müller cells endfeet (arrows). (b) Labelling of KAT II. KAT II immunoreactivity is preferentially localized on cells in the ganglion cell layer (arrows)

Discussion

CAm are the only light microscopically visible structures in the retina and optic nerve associated with degeneration and are still of mysterious nature. Up to now, only limited data are found in the literature concerning the mechanisms of their formation.

The present study is the first to demonstrate the immunoreactivity of KAT I and KAT II in CAm in the human retina and optic nerve. CAm expressing both enzymes were observed in all cases in the retina and in the prelaminary, laminary and retrolaminary regions of the optic nerve.

A variety of staining patterns of KAT I depending on the location of CAm were found. In general, there was more pronounced staining in the retrolaminar part of the optic nerve. Immunoreactivity of KAT II was less pronounced than KAT I, with no association of staining variety and localisation of CAm. By showing that all CAm expressing KATs are PAS--positive we were able to prove that KAT-stained structures are CAm. The findings in PAS-stained sections are in agreement with previous results of Kubota and colleagues [13].

It has been well documented that CAm have no pathognostic significance, but that they accumulate in certain conditions and pathological processes [15,17]. Numerous factors have been suggested to contribute to the formation of CAm, such as the components of the degraded cells, metabolites originated from the cerebrospinal fluid, blood and the mesenchyma of pia mater and adventitia of the vessel wall [14]. Importantly, hypoxic/ischaemic injury has been shown to potentiate the enigmatic biological pathway leading to the formation of CAm during ageing. The authors speculated that damaged mitochondria and de novo induced or overproduced proteins during cellular insults may be sequestrated by CAm [4]. Assuming that the formation of CAm represents an arrangement for the management of products escaping normal cell catabolism [5], greatly increased numbers of CAm in the optic nerve and retina may reflect increased metabolic work caused by repetitive cellular stress [4], and possibly the presence of KAT I and II in CAm might suggest a potential role of those enzymes in mechanisms of endogenous cellular protection against insult.

Interestingly, there are some data from brain studies suggesting that the CAm possess a relatively high affinity to accumulate to some extent 'protective' substances (such as Bcl2, AP1, heat shock proteins, etc.) which could rescue nerve cells from the devastating effects of ischaemia or ageing [4,6,9]. So far, immunohistochemical investigations have demonstrated anti-tau-2 immunoreactivity in CAm in the retina, optic nerve and brain tissue [16].

Only recently, the age-dependant decrease of cellular expression of both KAT I and II was observed in the retina of DBA/2J mice, a model for ocular hypertension [22]. Moreover, we have already shown that KYNA deficiency is causally related to the pathology of excitotoxic retinal diseases and that NMDA-induced retinal ganglion cell loss may cause alterations of KYNA content in the rat retina [25]. Importantly, there are data suggesting that alterations of KYNA synthesis are involved in the pathomechanisms of several brain disorders, e.g. Parkinson's disease [18], Huntington's disease [3], Alzheimer's disease [2] and epilepsy [32].

In vitro studies have revealed that KAT II is responsible for most of the KYNA formation in the brain, although changes in the relative importance of the two enzymes may occur in various pathophysiological situations [10]. Moreover, dysfunction of KYNA synthesis in the brain was suggested to be one of the factors contributing to neuronal degeneration [3,18,32]. It was reported that in several regions of Alzheimer's disease brain activity of KAT I was significantly increased while only a minor increase of KAT II was observed [2]. Since increased CAm formation was described in Alzheimer's disease brain [28] we speculate that it may explain stronger KAT I immunoreactivity in CAm as compared to KAT II, which was observed in the present study.

Only recently, KYNA content and enzymatic activities of its synthesising enzymes in the human retina and vitreous body have been characterized using biochemical methods [33]. Cellular expression of KATs in neurons and glial cells of CNS is well described [12,23,26,27]. The present study, similarly to results of our previous studies in rodents [22,23,26], showed that both KATs were present in the human retina. KAT I was preferentially localised on Müller cell endfeet while KAT II was expressed in cells within the ganglion cell layer. Interestingly, unequivocal representation of KAT I and II immunoreactivity in CAm demonstrated in this study may suggest extracellular expression of both enzymes or extracellular accumulation via a specific transport outside the cell body. The question arises whether presence of these proteins is a primary event in CAm formation or a secondary mechanism induced by some products of a degenerative process (ageing, neurodegeneration) or by recurrent functional disturbances of the cellular barriers. It might be hypothesised that the enzymes are released from cells dying due to degeneration and are accumulated in CAm. However, mechanisms leading to extracellular occurrence of both enzymes reported here need further investigations.

The presence of KATs in CAm in the human retina and optic nerve suggest that KYNA synthesis might be involved in the mechanisms of retinal ageing and neurodegeneration leading to CAm formation. Future extended experiments are necessary to provide a better understanding of the involvement of tryptophan metabolism in the development of degenerative retinal products, which also might help to understand the biological role and significance of CAm.

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References

1. Avendano J, Rodrigues MM, Hackett JJ, Gaskins R. Corpora amylacea of the optic nerve and retina: a form of neuronal degeneration. Invest Ophthalmol Vis Sci 1980; 19: 550-555.
2. Baran H, Jellinger K, Deecke L. Kynurenine metabolism in Alzheimer's disease. J Neural Transm 1999; 106: 165-181.
3. Beal MF, Matson WR, Storey E, Milbury P, Ryan EA, Ogawa T, Bird ED. Kynurenic acid concentrations are reduced in Huntington's disease cerebral cortex. J Neurol Sci 1992; 108: 80-87.
4. Botez G, Rami A. Immunoreactivity for Bcl-2 and C-Jun/AP1 in hippocampal corpora amylacea after ischaemia in humans. Neuropathol Appl Neurobiol 2001; 27: 474-480.
5. Cavanagh JB. Corpora-amylacea and the family of polyglucosan diseases. Brain Res Rev 1999; 29: 265-295.
6. Cisse S, Perry G, Lacoste-Royal G, Cabana T, Gauvreau D. Immunochemical identification of ubiquitin and heat-shock proteins in corpora amyleacea from normal aged and Alzheimer's disease brains. Acta Neuropathol 1993; 85: 233-240.
7. Cursiefen C, Rummelt C, Kuchle M. Immunohistochemical localization of vascular endothelial growth factor, transforming growth factor alpha, and transforming growth factor beta1 in human corneas with neovascularization. Cornea 2000; 19: 526-533.
8. Dolman CL, McCormick AQ, Drance SM. Aging of the optic nerve. Arch Ophthalmol 1980; 98: 2053-2058.
9. Gati I, Leel-Ossy L. Heat shock protein 60 in corpora amylacea. Pathol Oncol Res 2001; 7: 140-144.
10. Guidetti P, Okuno E, Schwarcz R. Characterization of rat brain kynurenine aminotransferases I and II. J Neurosci Res 1997; 50: 457-465.
11. Hilmas C, Pereira EF, Alkondon M, Rassoulpour A, Schwarcz R, Albuquerque EX. The brain metabolite kynurenic acid inhibits alpha7 nicotinic receptor activity and increases non-alpha7 nicotinic receptor expression: physiopathological implications. J Neurosci 2001; 21: 7463-7473.
12. Kapoor R, Okuno E, Kido R, Kapoor V. Immuno-localization of kynurenine aminotransferase (KAT) in the rat medulla and spinal cord. Neuroreport 1997; 8: 3619-3623.
13. Kubota T, Holbach LM, Naumann GO. Corpora amylacea in glaucomatous and non-glaucomatous optic nerve and retina. Graefes Arch Clin Exp Ophthalmol 1993; 231: 7-11.
14. Leel-Ossy L. New data on the ultrastructure of the corpus amylaceum (polyglucosan body). Pathol Oncol Res 2001; 7: 145--150.
15. Leel-Ossy L. The occurrence of corpus amylaceum (polyglucosan body) in diabetes mellitus. Neuropathology 1995; 15: 108-111.
16. Loeffler KU, Edward DP, Tso MO. Tau-2 immunoreactivity of corpora amylacea in the human retina and optic nerve. Invest Ophthalmol Vis Sci 1993; 34: 2600-2603.
17. Lowe J, Mayer RJ, Landon M. Ubiquitin in neurodegenerative diseases. Brain Pathology 1993; 3: 55-65.
18. Ogawa T, Matson WR, Beal MF, Myers RH, Bird ED, Milbury P, Saso S. Kynurenine pathway abnormalities in Parkinson's disease. Neurology 1992; 42: 1702-1706.
19. Okuno E, Du F, Ishikawa T, Tsujimoto M, Nakamura M, Schwarcz R, Kido R. Purification and characterization of kynurenine-pyruvate aminotransferase from rat kidney and brain. Brain Res 1990; 534: 37-44.
20. Okuno E, Tsujimoto M, Nakamura M, Kido R. 2-Aminoadipate--2-oxoglutarate aminotransferase isoenzymes in human liver: a plausible physiological role in lysine and tryptophan metabolism. Enzyme Protein 1993; 47: 136-148.
21. Perkins MN, Stone TW. An iontophoretic investigation of the actions of convulsant kynurenines and their interaction with the endogenous excitant quinolinic acid. Brain Res 1982; 247: 184-187.
22. Rejdak R, Kohler K, Kocki T, Shenk Y, Turski WA, Okuno E, Lehaci C, Zagorski Z, Zrenner E, Schuettauf F. Age-dependent decrease of retinal kynurenate and kynurenine aminotransferases in DBA/2J mice, a model of ocular hypertension. Vision Res 2004; 44: 655-660.
23. Rejdak R, Shenk Y, Schuettauf F, Turski WA, Okuno E, Zagorski Z, Zrenner E, Kohler K. Expression of kynurenine aminotransferases in the rat retina during development. Vision Research 2004; 44: 1-7.
24. Rejdak R, Zarnowski T, Turski WA, Kocki T, Zagorski Z, Guenther E, Kohler K, Zrenner E. Changes of kynurenic acid content in the rat and chicken retina during ontogeny. Graefes Arch Clin Exp Ophthalmol 2002; 240: 687-691.
25. Rejdak R, Zarnowski T, Turski WA, Kocki T, Zagorski Z, Zrenner E, Schuettauf F. Alterations of kynurenic acid content in the retina in response to retinal ganglion cell damage. Vision Res 2003; 43: 497-503.
26. Rejdak R, Zarnowski T, Turski WA, Okuno E, Kocki T, Zagorski Z, Kohler K, Guenther E, Zrenner E. Presence of kynurenic acid and kynurenine aminotransferases in the inner retina. Neuroreport 2001; 12: 3675-3678.
27. Rejdak R, Zielinska E, Shenk Y, Turski WA, Okuno E, Zarnowski T, Zagorski Z, Zrenner E, Kohler K. Ontogenic changes of kynurenine aminotransferase I activity and its expression in the chicken retina. Vision Res 2003; 43: 1513-1517.
28. Renkawek K, Bosman GJ. Anion exchange proteins are a component of corpora amylacea in Alzheimer disease brain. Neuroreport 1995; 6: 929-932.
29. Stone TW. Development and therapeutic potential of kynurenic acid and kynurenine derivatives for neuroprotection. Trends Pharmacol Sci 2000; 21: 149-154.
30. Turski WA, Nakamura M, Todd WP, Carpenter BK, Whetsell WO Jr, Schwarcz R. Identification and quantification of kynurenic acid in human brain tissue. Brain Res 1988; 454: 164-169.
31. Woodford B, Tso MO. An ultrastructural study of the corpora amylacea of the optic nerve head and retina. Am J Ophthalmol 1980; 90: 492-502.
32. Yamamoto H, Murakami H, Horiguchi K, Egawa B. Studies on cerebrospinal fluid kynurenic acid concentrations in epileptic children. Brain Dev 1995; 17: 327-329.
33. Zarnowski T, Rejdak R, Zagorski Z, Juenemann AG, Zrenner E, Kocki T, Urbanska EM, Turski WA. Content of kynurenic acid and activity of kynurenine aminotransferases in mammalian eyes. Ophthalmic Res 2004; 36: 124-128.