Original Paper

NEURO-SIGNALS

Neurosignals 2005;14:126–135
DOI: 10.1159/000086295

Received: November 24, 2004
Accepted after revision: March 10, 2005

Age-Related Increase of Kynurenic Acid in Human Cerebrospinal Fluid – IgG and β_2-Microglobulin Changes

Berthold Kepplinger[a, b] Halina Baran[d] Astrid Kainz[a, b] Heber Ferraz-Leite[e]
Jea Newcombe[f] Pavol Kalina[b, c]

[a] Department of Neurology, Klinikum Mostviertel Amstetten, General Hospital, Amstetten, [b] Department of Neurology, [c] Neuroradiological Division, Diagnostic and Therapy Centre, Ostarrichiklinikum, Neuropsychiatric Hospital, Mauer/Amstetten, [d] Pharmacology and Toxicology, Veterinary Medical University Vienna, [e] University Clinic of Neurosurgery, Vienna University Hospital AKH, Vienna, Austria; [f] NeuroResource, Institute of Neurology, University College London, London, UK

Key Words
Ageing · Cerebrospinal fluid · Kynurenic acid · IgG · β_2-Microglobulin

Abstract

Kynurenic acid (KYNA) is an endogenous metabolite in the kynurenine pathway of tryptophan degradation and is an antagonist at the glycine site of the N-methyl-*D*-aspartate as well as at the alpha 7 nicotinic cholinergic receptors. In the brain tissue KYNA is synthesised from *L*-kynurenine by kynurenine aminotransferases (KAT) I and II. A host of immune mediators influence tryptophan degradation. In the present study, the levels of KYNA in cerebrospinal fluid (CSF) and serum in a group of human subjects aged between 25 and 74 years were determined by using a high performance liquid chromatography method. In CSF and serum KAT I and II activities were investigated by radioenzymatic assay, and the levels of β_2-microglobulin, a marker for cellular immune activation, were determined by ELISA. The correlations between neurochemical and biological parameters were evaluated. Two subject groups with significantly different ages, i.e. <50 years and >50 years, $p < 0.001$, showed statistically significantly different CSF KYNA levels, i.e. 2.84 ± 0.16 fmol/μl vs. 4.09 ± 0.14 fmol/μl, $p < 0.001$, respectively; but this difference was not seen in serum samples. Interestingly, KYNA is synthesised in CSF principally by KAT I and not KAT II, however no relationship was found between enzyme activity and ageing. A positive relationship between CSF KYNA levels and age of subjects indicates a 95% probability of elevated CSF KYNA with ageing ($R = 0.6639$, $p = 0.0001$). KYNA levels significantly correlated with IgG and β_2-microglobulin levels ($R = 0.5244$, $p = 0.0049$; $R = 0.4253$, $p = 0.043$, respectively). No correlation was found between other biological parameters in CSF or serum. In summary, a positive relationship between the CSF KYNA level and ageing was found, and the data would suggest age-dependent increase of kynurenine metabolism in the CNS. An enhancement of CSF IgG and β_2-microglobulin levels would suggest an activation of the immune system during ageing. Increased KYNA metabolism may be involved in the hypofunction of the glutamatergic and/or nicotinic cholinergic neurotransmission in the ageing CNS.

KARGER
Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2005 S. Karger AG, Basel
1424–862X/05/0143–0126$22.00/0

Accessible online at:
www.karger.com/nsg

Prof. H. Baran, PhD
Pharmacology and Toxicology, Veterinary Medical University Vienna
Veterinärplatz 1
AT–1210 Vienna (Austria)
Tel. +43 1 25077 4519, Fax +43 1 25077 4590, E-Mail Halina.Baran@vu-wien.ac.at

Introduction

Glutamate is an important metabolic agent in the mammalian central nervous system (CNS), and its excitotoxic activity has been proposed to contribute to the pathogenesis of various CNS disorders in humans [1–3]. Kynurenic acid (KYNA) is a well-known endogenous antagonist of the glutamate ionotropic excitatory amino acid receptors N-methyl-*D*-aspartate (NMDA), alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid and kainate [4] and of the nicotine cholinergic subtype alpha 7 receptors [5], and KYNA neuroprotective and anticonvulsive activities have been demonstrated in animal models of neurodegenerative diseases [6, 7]. Because of KYNA's neuromodulatory character, its involvement has been speculatively linked to the pathogenesis of a number of neurological conditions including those in the ageing process.

Different patterns of abnormalities in various stages of KYNA metabolism in the CNS have been reported in Alzheimer's disease [8], Parkinson's disease [9] and Huntington's disease [10–12]. In HIV-1-infected patients [13] and in patients with Lyme neuroborreliosis [14] a marked rise of KYNA metabolism was seen.

In the ageing process KYNA metabolism in the CNS of rats shows a characteristic pattern of changes throughout the life span [15–18]. A marked increase of the KYNA content in the CNS occurs before the birth, followed by a dramatic decline on the day of birth [16]. A low activity was seen during ontogenesis [15], and a slow and progressive enhancement occurs during maturation [15] and ageing [17]. This remarkable profile of KYNA metabolism alterations in the mammalian brain has been suggested to result from the development of the organisation of neuronal connections and synaptic plasticity, development of receptor recognition sites, maturation and ageing [4].

There is significant evidence that KYNA can improve cognition and memory [19], but it has also been demonstrated that it interferes with working memory [20]. Impairment of cognitive function in various neurodegenerative disorders is accompanied by profound reduction and/or elevation of KYNA metabolism. The view that enhancement of CNS KYNA levels could underlie cognitive decline is supported by the increased KYNA metabolism in Alzheimer's disease [8], by the increased KYNA metabolism in Down's syndrome [21] and the enhancement of KYNA function during the early stage of Huntington's disease [11]. In 1999, Baran et al. [8] suggested that the blocking of the glutamatergic neurotransmission in Alzheimer's disease patients due to increased CNS KYNA levels could be involved in memory and cognition impairments. Since KYNA can block both glutamatergic [4] and the alpha 7 nicotinic cholinergic activities [5], it is reasonable to speculate that the fluctuation of KYNA metabolism could significantly influence both. The potency of KYNA as a nicotinic antagonist is similar to its potency as an antagonist at the glycine site of NMDA receptors [22]. The recent memory deficit and/or hypofunction of neurotransmission [3] and hyperfunction of KYNA [8] may reflect as a key event, which could share several important elements in dementia of Alzheimer and ageing as well.

L-kynurenine is the primary metabolite of the enzymatic degradation of tryptophan by indoleamine-2,3-dioxygenase (IDO). An interaction between glial and immune cells through the release of cytokines has been described in many pathological conditions, and the secretion of cytokines, such as interferon-γ, interleukin-1 and 6, tumor necrosis factor-α, in response to injury and infection play a prominent role in the initiation and maintenance of neurotoxic immune responses within the injured CNS and further propagate CNS damage [23–26]. Activation of IDO by interferon-γ has been observed in human monocytes/macrophages and a variety of human cells and cell lines in vitro [27, 28]. β_2-Microglobulin, a marker of activation of immune cells, is a small protein associated with the class I major histocompatibility complex antigen. Increasing β_2-microglobulin levels are considered to reflect an activation of the cellular immune system and enhancement in cell membrane turnover [29, 30]. Several disorders of the CNS are associated with increased cerebrospinal fluid (CSF) β_2-microglobulin levels, such as in Alzheimer type dementia [31], brain infarct and meningitis [32] and HIV infection [33]. A positive correlation between increase of kynurenine metabolites and β_2-microglobulin levels in CSF and serum has been reported in HIV type 1 infection [13]. In the present study we asked whether KYNA metabolism and markers of the immune system undergo alterations in the CSF and serum of human subjects without neurological disease but with advancing age. We analysed the correlation between changes of KYNA metabolism and biological parameters in CSF and serum in order to find indications for relationships between KYNA levels and ageing and immune markers involvement.

Materials and Methods

Materials

L-kynurenine, KYNA and pyridoxal-5′-phosphate were purchased from Sigma. $[^3H]L$-kynurenine (specific activity, 41 Ci/mmol) was supplied by Amersham, England. Rabbit anti-human IgG, rabbit anti-human IgM and rabbit anti-human albumin were obtained from Dade Behring, Turbiquant Germany. The β_2-Microglobulin ELISA KIT was obtained from Instrumentation Laboratory GmbH, Austria. All other chemicals used were of the highest commercially available purity.

Subjects

Out of a larger series of acute headache patients who underwent lumbar puncture to exclude subarachnoidal haemorrhage or viral or bacterial meningitis, 27 individuals were selected for this study because CSF samples from these patients did not contain erythrocytes, and no abnormalities in neuroimaging and further clinical investigations, which included in most cases electroencephalography and transcranial Doppler sonography, were found. All of these investigated individuals were not treated with analgesic drugs prior to the lumbar puncture and following investigation confirmed a normal clinical status. Ages ranged between 25 and 74 years. Lumbar puncture was carried out to obtain CSF for routine parameter determinations, as e.g. number of erythrocytes, cell count, protein content and detection of IgG oligoclonal bands, autochtonic immunoglobulin production IgG index and albumin content. Blood samples were taken for routine investigation of leukocyte count, IgG and IgM and albumin content. For neurochemical analyses samples of CSF and serum were collected immediately in 1-ml aliquots and stored at –30°C until analysed. CSF and serum were coded to make anonymous and the study was carried out according to the ethical regulations of Lower Austria.

Investigations of the Biological Parameters

Measurement of protein, albumin, IgG, IgM and white blood cell counts were carried out using routine laboratory methods. CSF:serum IgG ratio and CSF:serum albumin ratio and the IgG index were calculated [34]. For determination of oligoclonal IgG bands, agarose isoelectric focusing was performed, followed by transfer to cellulose nitrate membrane and double antibody avidin-biotin-peroxidase labelling [35].

Neuroradiological Investigations

Routine clinical investigations of cranial computer tomography and magnetic resonance tomography with computer tomography or MR angiography of cerebral vessels, and electroencephalography and transcranial Doppler sonography were carried out.

Measurement of KYNA

The measurement of KYNA was performed according to Shibata [36] and Swartz et al. [37] with modification described by Baran et al. [8]. Briefly, CSF or serum samples were mixed with 0.2 *M* HCl (vol/vol) and centrifuged (20 min, 14,000 rpm). The supernatant was applied to a Dowex 50W cation exchange column pre-washed with 0.1 *M* HCl. Subsequently, the column was washed with 0.1 *M* HCl and 1 ml distilled water, and KYNA was eluted with 2 ml distilled water [38] and was quantitated by high performance liquid chromatography system coupled with fluorescence detection.

Measurement of Kynurenine Aminotransferases I and II in CSF and Serum

Measurement of of kynurenine aminotransferases (KAT) I and II activities in CSF and serum was performed using a radioenzymatic assay described by Schmidt et al. [39]. In brief, the reaction mixture contained CSF or serum, 100 μ*M* 1.175 μCi/μmol $[^3H]L$-kynurenine, 1 m*M* pyruvate, 70 μ*M* pyridoxal-5′-phosphate and 150 m*M* 2-amino-2-methyl-l-propanol buffer pH 9.6 for KAT I in a total volume of 200 μl. For KAT II activity measurement 150 m*M* Tris-acetate buffer pH 7.0 was used. The measurement of KAT II activity was performed in the presence and absence of 5 m*M* *L*-glutamine. After incubation for 16 h at 37°C, the reaction was stopped by adding 14 μl of 50% trichloroacetic acid and 1 ml of 0.1 *M* HCl. Denatured proteins were removed by centrifugation, and the synthesised $[^3H]$KYNA was purified on Dowex 50W cation exchange column [38] and quantified by liquid scintillation spectrometry. Blanks were prepared by boiling CSF or serum samples for 15 min before adding the reaction mixture.

Measurement of β_2-Microglobulin in CSF and Serum

β_2-Microglobulin concentration in CSF and serum was measured by using a commercial sandwich ELISA method.

Statistical Analysis

All mean values ± SEM are given. For statistical significance the one-way ANOVA and Student's t test were applied. Linear regression analysis was performed using the least squares method. Correlation between clinical parameters, e.g. CSF IgG, IgG index, CSF KYNA levels, serum KYNA levels and KAT activities with ageing was analysed. The levels for statistical significance were taken as $p < 0.05$.

Results

Biological Parameters

Two groups of human subjects of significantly different ages (age <50 and age >50) were evaluated. The mean age of the groups was 35.4 ± 2.2 years, ranging from 25 to 50 years, and 61.6 ± 3.3 years, ranging from 50 to 74 years, and the difference between both groups was significant, i.e. $p < 0.001$ (table 1). The values of CSF IgG, the CSF:serum IgG ratio and β_2-microglobulin in CSF were significantly higher in subjects of the >50 years age group (table 1). A moderate increase of protein levels in CSF and of the CSF:serum albumin ratio was observed of the >50 years age group. No significant differences regarding parameters could be found in the serum.

Correlation between Biological Parameters

Using a linear regression analysis it could be observed that with increasing age of the subjects the CSF IgG values also increased significantly (fig. 1; R = 0.4582, p = 0.0146). However, no positive correlation was found between IgG index and advancing age (R = 0.2988, p =

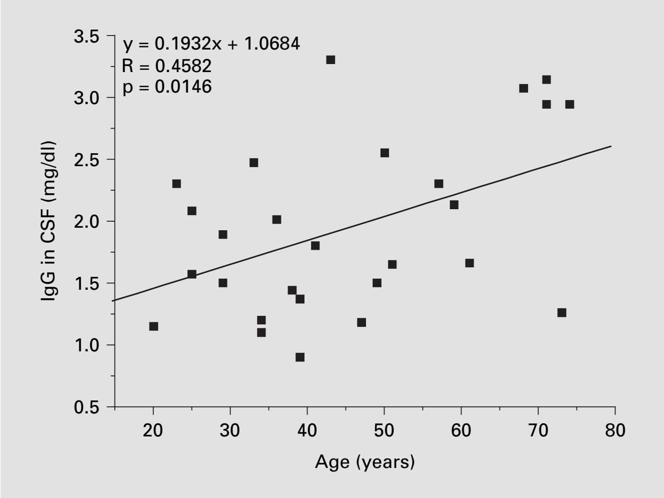

Fig. 1. Linear regression analysis between CSF IgG values and age in human subjects.

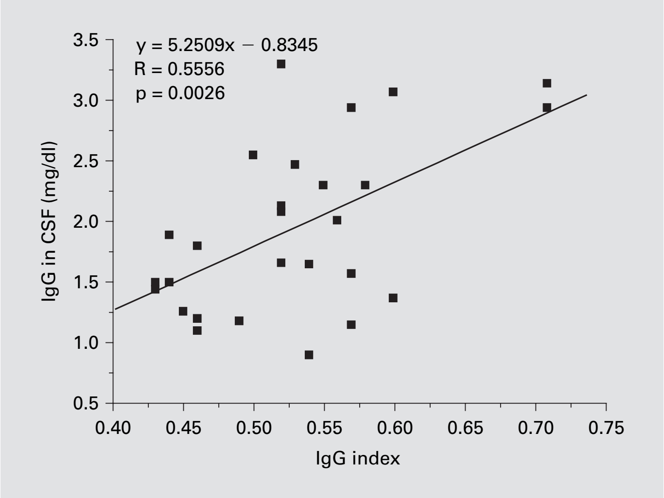

Fig. 2. Linear regression analysis between CSF IgG values and IgG index in human subjects.

Table 1. Biological parameters of subjects

Biological parameters	Age, years			Age >50, % of age <50
	25–74 (n = 27)	<50 (n = 17)	>50 (n = 10)	
Number of leukocytes, 10^6/l	6.49 ± 0.37	6.62 ± 0.40	6.26 ± 0.78	95
IgG serum, mg/dl	923.7 ± 49.7	940.9 ± 71.0	891.4 ± 56.8	95
IgM serum, mg/dl	81.1 ± 7.0	84.9 ± 8.6	74.0 ± 12.6	87
Proteins in CSF, mg/dl	33.9 ± 1.7	32.2 ± 2.1	36.8 ± 3.0	114
Cell count, × 10^6/l	2.71 ± 0.24	2.51 ± 0.26	2.91 ± 0.22	116
Oligoclonal IgG bands	negative	negative	negative	100
IgG CSF, mg/dl	1.94 ± 0.13	1.69 ± 0.15	2.36 ± 0.21**	140
Ratio CSF:serum IgG	2.15 ± 0.17	1.89 ± 0.21	2.58 ± 0.24*	137
Ratio CSF:serum albumin	4.00 ± 0.26	3.66 ± 0.35	4.59 ± 0.30	125
IgG index	0.524 ± 0.013	0.506 ± 0.014	0.554 ± 0.022	109
CSF β_2-Microglobulin in CSF, mg/l	1.20 ± 0.08	1.02 ± 0.39 (14)	1.47 ± 0.14** (9)	144
β_2-Microglobulin in serum, mg/l	3.01 ± 0.27	3.14 ± 0.32 (16)	2.73 ± 0.53 (7)	87
Age, years	45.1 ± 3.0	35.4 ± 2.2	61.6 ± 3.3***	174
Females/males	12/15	7/10	5/5	

Data represent mean ± SEM. Number of subjects is given in parentheses.
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs. group with age <50 years using Student's t test.

0.1301). Subsequently, significant correlation was obtained between the value of CSF IgG and the IgG index (fig. 2; R = 0.5556, p = 0.0026). A moderate positive relationship was found between the CSF:serum albumin ratio and age and between CSF:serum IgG ratio and age (R = 0.3644, p = 0.0873; R = 0.3552, p = 0.0690, respectively).

KYNA Levels in CSF and Serum

In all subjects aged from 25 to 74 years the mean value of KYNA levels in CSF and in the serum was 3.30 ± 0.17 and 26.54 ± 1.87 fmol/μl, respectively. In the >50 years age group the level of CSF KYNA was significantly higher ($p < 0.001$) than in subjects of the age <50 years, but no differences could be found in the sera (fig. 3). With-

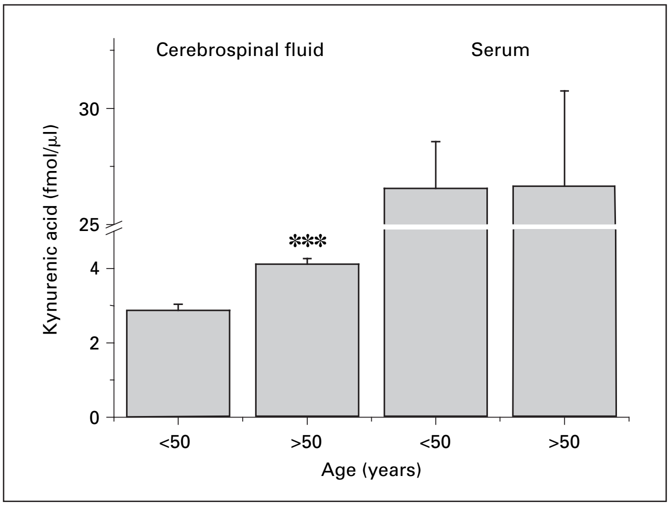

Fig. 3. KYNA in CSF and serum in two subject groups, i.e. age <50 years (n = 17) and age >50 years (n = 10). Significance between both groups was *** $p < 0.0001$.

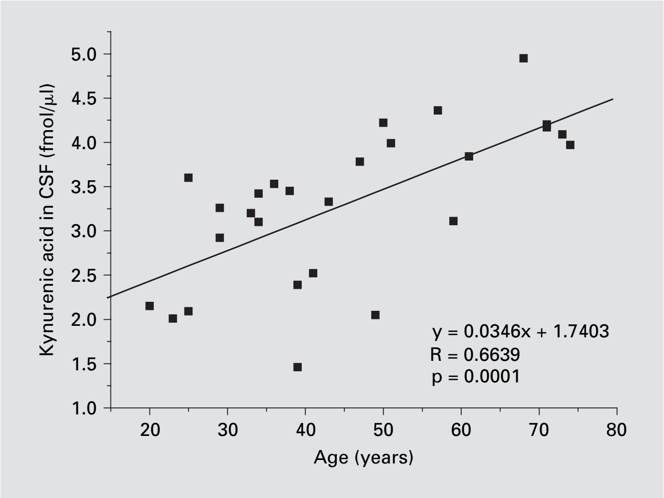

Fig. 4. Linear regression analysis between CSF KYNA concentration and age in human subjects.

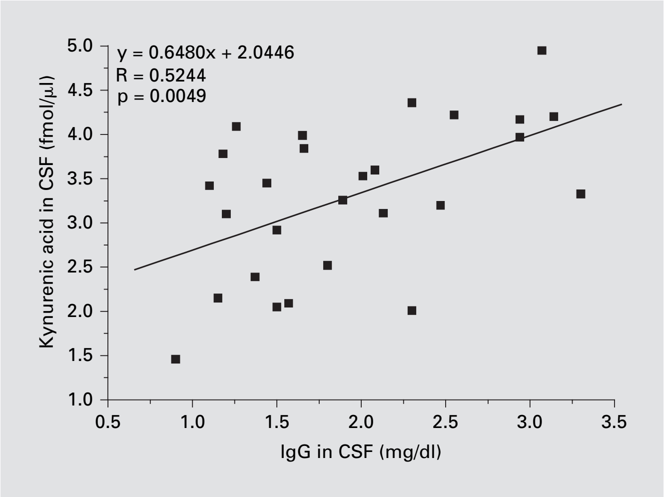

Fig. 5. Linear regression analysis between CSF KYNA concentration and CSF IgG level in human subjects.

in each group no influence of sex on CSF KYNA levels was found.

Kynurenine Aminotransferase I and II

Using a radioenzymatic assay we measured formation of KYNA from *L*-kynurenine in CSF and less in serum. Using assay condition for KAT I and II the conversion of *L*-kynurenine to KYNA was 155.2 ± 20.3 and 19.2 ± 4.3 KYNA fmol/µl/h, respectively. However, in 9 CSF samples of 27 no formation of KYNA (negative value) was seen using KAT I reaction conditions, and in remaining samples the results ranged between 35 and 346.5 KYNA fmol/µl/h. The KAT II activity in CSF ranged between 2.4 and 34.0 KYNA fmol/µl/h and in 19 samples no formation of KYNA (negative value) was measured. In the presence of 5 m*M* glutamine KAT II activity was moderately reduced to 15.4 ± 2.9 KYNA fmol/µl/h. The value of KAT II in the presence of 5 m*M* glutamine ranged between 5.0 and 29.3 KYNA fmol/µl/h and in 17 CSF probes no formation was seen (negative value). Using assay condition for KAT I and II, in all serums the conversion was less than 2 fmol/µl/h or there was no formation of KYNA.

Correlation between Kynurenine Metabolism and Biological Parameters

A positive relationship was found between CSF KYNA levels and advancing age, indicating a 95% probability of increasing CSF KYNA levels with age (fig. 4; $R = 0.6639$, $p < 0.0001$). No positive relationship between both parameters was measured in the serum ($R = 0.1118$, $p = 0.6114$). A comparison between CSF KYNA and CSF IgG levels revealed a significantly positive relationship (fig. 5; $R = 0.5244$, $p = 0.0049$). No positive correlation was seen between CSF KYNA levels and the IgG index ($R = 0.1898$, $p = 0.343$), between CSF KYNA levels and ratio serum:CSF IgG ($R = 0.3021$, $p = 0.1257$) and be-

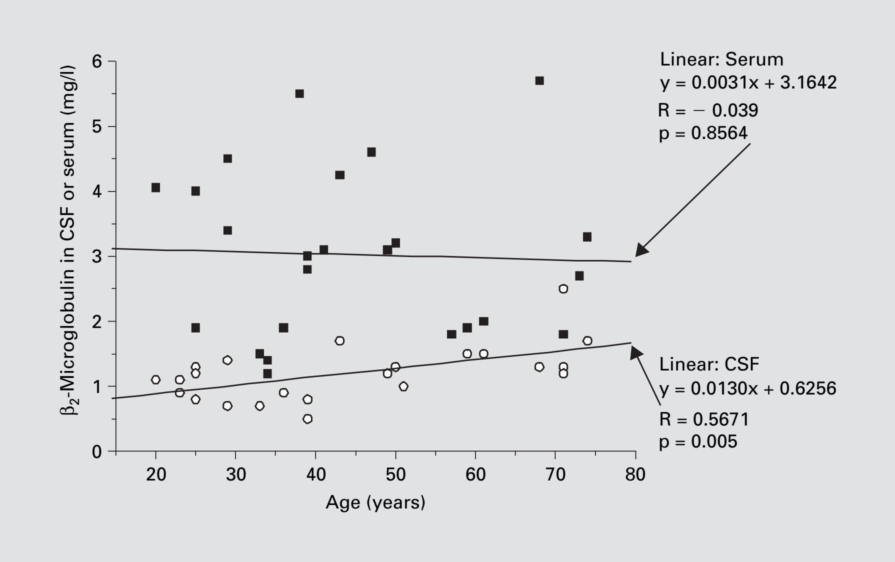

Fig. 6. Correlation between CSF β_2-microglobulin levels and age (○), and lack of correlation between serum β_2-microglobulin levels and age (■) in human subjects.

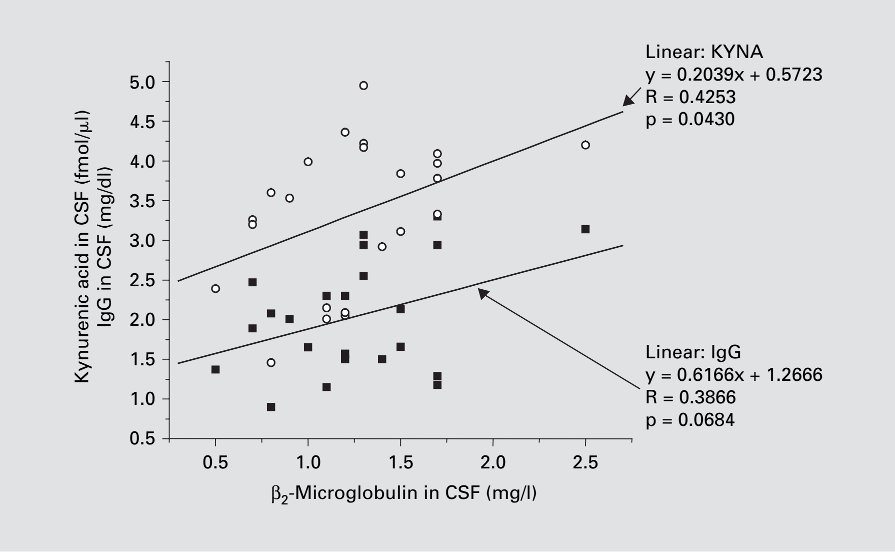

Fig. 7. Correlation between CSF KYNA and CSF β_2-microglobulin concentrations (○) and between CSF IgG and CSF β_2-microglobulin levels (■) in human subjects.

tween CSF KYNA and ratio serum:CSF albumin (R = 0.3057, p = 0.1210). No correlation could be found between KAT activities and biological parameters.

In the CSF but not in the serum significant differences between two subject groups (age <50 vs. age >50) could be observed regarding the β_2-microglobulin level (table 1). A positive correlation was found between CSF β_2-microglobulin and ageing, whereas no correlation was seen between serum β_2-microglobulin and ageing (fig. 6; R = 0.5671, p = 0.005 and R = –0.039, p = 0.8564), respectively. Furthermore, a positive relationship was found between CSF KYNA and β_2-microglobulin levels, and a moderate relationship was found between CSF IgG and β_2-microglobulin concentrations (fig. 7; R = 0.4253, p = 0.0430 and R = 0.3866, p = 0.0684, respectively). No positive relationship was found between serum β_2-microglobulin and serum IgG (R = 0.357, p = 0.0939), between serum β_2-microglobulin and serum IgM (R = 0.0327, p = 0.8823) and between serum β_2-microglobulin and serum KYNA levels (R = 0.1256, p = 0.5679).

Discussion

The most notable finding of the study is that in the CSF of human subjects without detectable neurological disease, the KYNA level significantly increase with advancing age. In addition, with advancing age, the levels of immunoreactive markers IgG and β_2-microglobulin were increasing, whereas no increase of KYNA, β_2-microglobulin or IgG was found in the serum. Interestingly, Heyes et al. [40] observed higher CSF KYNA levels (3.49 ± 0.44 vs. 2.23 ± 0.28 n*M*) and higher CSF *L*-kynurenine levels (52.9 ± 3.1 vs. 33.2 ± 2.8 n*M*) in older control subjects (59.1 ± 14.2 vs. 35.2 ± 8.4 years, respectively). Furthermore, in line with our data, another research group has found that in childhood levels of CSF IgG also were significantly different from adult reference readings [41]. The increase of CSF KYNA with ageing has been reported in sheep as well [42]. Revealed data would suggest the age-related increase of KYNA in human CSF and the involvement of increased immune markers.

Recently, Erhardt et al. [43] demonstrated increased CSF KYNA with ageing in schizophrenia patients, but no correlation was found in healthy volunteers, probably due to a narrow age range (between 22 and 44 years) of healthy volunteers investigated. The mean of CSF KYNA values of subjects investigated in the present study was 3.3 ± 0.17 n*M*. Accumulated data on CSF KYNA levels in healthy volunteers/control subjects indicate a wide variation of concentration (between 0.67 and 4.09 n*M*) [7, 40, 43, 44]. Several previously published papers suggested a connection between increase of CSF KYNA and pain [37, 45]. Indeed, Swartz et al. [37] found high CSF KYNA levels in patients with fever or headache (5.09 ± 1.04 n*M*). Interestingly, analgesic drugs are also able to modify the KYNA concentration; however, the mechanism of increasing KYNA levels due to pain and its action to modulate pain needs to be more clarified [46].

KYNA content measured in CSF depends on differently regulated events involved in KYNA metabolism, such as substrate availability, uptake, its formation and release into CSF and diffusion into the blood [4, 18, 38, 47, 48]. In adult animals KYNA does not or very slightly penetrates through the blood-brain barrier (BBB) [49]. With increasing age of the subjects analysed, a moderate increase of BBB permeability was observed in line with previously published data [50], and a slightly positive relationship between CSF:serum albumin concentrations ratio and ageing or CSF KYNA levels would not exclude a very moderate diffusion of KYNA into the aged CNS. Since no difference in KYNA serum level was found in subjects investigated, we would suggest that CSF KYNA increases with advancing age originate from the brain. In this connection, in the rat brain but not in the rat liver, the KYNA metabolism was significantly increased with advanced age, as well [17].

In the CNS, KYNA may derive from *L*-kynurenine that was synthesised either by CNS tissue following IDO, the first enzyme of the kynurenine pathway in extrahepatic tissue, or from *L*-kynurenine that had entered from blood [51]. Interestingly, the *L*-kynurenine was shown to be significantly higher in the CSF of healthy aged rats [52] and in older human control subjects too [40]. Furthermore, those authors [51] have also demonstrated that the ratio of *L*-kynurenine:tryptophan was doubled in aged rats (28–32 months), comparing to mature rats (4–6 months), and suggested age-related activation of IDO activities. Similar changes might occur in the human brain, for example due to the induction of enzyme IDO activities. Human macrophages express IDO activities, and the enzyme is sensitive to interferon-γ [53]. A significant increase of kynurenine pathway enzyme activities due to interferon-γ stimulation was demonstrated in blood macrophages, astrocytes, neurones and also B lymphocyte [54]. No data are currently available about interferon-γ formation in the human CNS during the ageing process.

In the human brain KYNA is synthesised from *L*-kynurenine by KAT I and KAT II [39, 55, 56]. Both proteins show distinct catalytic properties, e.g. KAT I has a pH optimum of 9.5–10, while KAT II displays a pH optimum of 7.4 [39, 55, 56]. In contrast to KAT II, KAT I shows particular preferences for amino acceptors and is inhibited by millimolar concentrations of *L*-amino acids, e.g. *L*-glutamine, *L*-phenylalanine and *L*-tryptophan [39, 56]. The cellular localisation of human brain KAT I and KAT II has not been demonstrated so far, whereas in the rat brain KAT antibodies used in immunohistological studies demonstrated a preferential astrocyte localisation of the protein [57].

Interestingly, in animal studies using in situ hybridisation it has been shown recently that the KAT I mRNA activity is expressed not only in the mitochondria of neurone and glial cells but also in the cytosol of the choroid plexus epithelial cells [58]. It is therefore reasonable to believe that the synthesis of KYNA also may take place in the choroid plexus due to KAT I activity, and synthesised KYNA is released into CSF and/or speculatively due to changes of the choroid plexus membrane the KAT proteins (or cells) from cytosol could easily diffuse into CSF. Indeed, in our previous [59] and in present study we measured in some CSF probes a moderate formation of KYNA, due to KAT I activity, but in some we found a negative value. Human KAT I is sensitive to amino acids [39, 56] and KAT I inhibition in the CSF was seen in the presence of 5 m*M* glutamine. In human, the CSF amino acids levels are in micromolar range, and no increase was observed in aged brain [60]. There could not be found any correlations between CSF KAT activities and other parameters.

An important pathological feature of a variety of neurological disorders, including the normal ageing process of the brain, is the activation of microglia. It has been suggested that the activation of microglia in white matter with advancing age may play a substantial role in the pathogenesis of normal brain ageing [61]. The increases of KYNA in the CSF during ageing could take place due to elevated KATs expression/activities in microglia and astrocytes as well. The latter cells are also immunoresponsive within the CNS [57]. Immunohistopathological studies indicate a predominantly astrocytic localisation of KAT in rat brains [57] and the major constituent of intermediate filaments in astrocytes, glial fibrillary acidic protein is increased with progressing age [62]. Astrocytes provide a variety of endogenous signals and diffusible factors that may serve to induce the formation of tight junctions, the expression of various proteins, maintain overall BBB integrity and promote differentiation and maturation of microglia [26, 63]. A large body of evidence now exists which implicates excessive microglia activation and proliferation in the development of neuronal death in various pathological disease states and in ageing process [63, 64]. At least in animal studies, experiments on KYNA synthesis with neuron-depleted rat brain tissue, which exhibits markedly elevated KYNA formation at a time of pronounced astrogliosis, suggest that astroglia are responsible for de novo formation of brain KYNA [38].

Interestingly, Morgan et al. [65] showed that food restriction decreased the transcription of glial fibrillary acidic protein in ageing rats and lowered microglia activation during ageing. A sparing of spatial memory can be achieved with applied diet restriction and the effect of ageing on NMDA receptor is associated with age-related declines in spatial memory [66]. The increases of IgG and β_2-microglobulin levels in the CSF during ageing can occur due to immune system activation. A positive relationship was found between both parameters. The age-related increase of CSF KYNA levels, IgG content and β_2-microglobulin concentration is of particular interest with respect to the ability to block NMDA and cholinergic alpha 7 receptors by drugs that activate tryptophan metabolism. In this connection, the CSF β_2-microglobulin level is significantly higher in Alzheimer's disease patients [31]. The binding site of the NMDA receptor antagonist is significantly reduced in 30-month-old mice, which indicates an age-related decline of neuronal response [66–68]. KYNA not only blocks the NMDA receptors [4], but also the alpha 7 subtype of nicotine cholinergic receptors [5]. The increase of CSF KYNA with ageing and the increase of KYNA metabolism in the CNS of Alzheimer's disease patients [8] would suggest a long-lasting blockade of glutamatergic and cholinergic neurotransmission, events that are involved in the pathogenesis of memory and cognitive impairments [3]. Therefore, the activation of tryptophan/kynurenine metabolism in aged persons and Alzheimer's disease patients may result in a negative response(s). An alteration of the receptor activities due to the ageing process may involve changes in KYNA metabolism and probably both processes could reciprocate.

In summary, increases in CSF KYNA, IgG and β_2-microglobulin levels have been identified in subjects of advanced age, and the data obtained would suggest the hypoglutamatergic and hypocholinergic stages in aged brains. Further studies need to be accomplished in order to provide more information on the mechanism(s) of direct and/or indirect KYNA action and its involvement in the memory and cognition impairment during ageing. The relationship between KYNA changes and activation of IgG and β_2-microglobulin levels with ageing is not yet clear.

Acknowledgements

This work is supported by Multiple Sklerosis Forschungsgesellschaft Wien (B.K.) and in part by Austrian 'Fonds der Wissenschaftlichen Forschung', project No. 15371 (H.B.).

References

1 Choi DW: Glutamate neurotoxicity and diseases of the nervous system. Neuron 1988;1:623–634.

2 Meldrum B, Garthwaite J: Excitatory amino acid neurotoxicity and neurodegenerative disease. Trends Pharmacol Sci 1990;11:379–387.

3 Bartus RT: On neurodegenerative diseases, models, and treatment strategies: Lessons learned and lessons forgotten a generation following the cholinergic hypothesis. Exp Neurol 2000;163:495–529.

4 Stone TW: Neuropharmacology of quinolinic and kynurenic acids. Pharmacol Rev 1993;45:309–379.

5 Hilmas C, Pereira EFR, 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.

6 Foster AC, Vezzani A, French ED, Schwarcz R: Kynurenic acid blocks neurotoxicity and seizures induced in rats by the related brain metabolite quinolinic acid. Neurosci Lett 1984;48:273–278.

7 Stone TW: Kynurenines in the CNS: From endogenous obscurity to therapeutic importance. Prog Neurobiol 2001;64:185–218.

8 Baran H, Jellinger K, Deecke L: Kynurenine metabolism in Alzheimer's disease. J Neural Transm 1999;106:165–181.

9 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.

10 Beal MF, Matson WR, Swartz KJ, Gamache PH, Bird ED: Kynurenine pathway measurements in Huntington's disease striatum. Evidence for reduced formation of kynurenic acid. J Neurochem 1990;55:1327–1339.

11 Connick JH, Carla V, Moroni F, Stone TW: Increase in kynurenic acid in Huntington's disease motor cortex. J Neurochem 1989;52:958–987.

12 Jauch D, Urbanska EM, Guidetti P, Bird ED, Vonsattel JPG, Whetsell Jr WO, Schwarcz R: Dysfunction of brain kynurenic acid metabolism in Huntington's disease: Focus on kynurenine aminotransferases. J Neurol Sci 1995;130:39–47.

13 Heyes MP, Brew BJ, Saito K, Quearry BJ, Price RW, Lee K, Bhalla RB, Der M, Markey SP: Inter-relationships between quinolinic acid, neuroactive kynurenines, neopterin and β_2-microglobulin in cerebrospinal fluid and serum of HIV-1-infected patients. J Neuroimmunol 1992;40:71–80.

14 Fuchs D, Dotevall L, Hagberg L, Werner ER, Wachter H: Kynurenine in cerebrospinal fluid of patients with Lyme neuroborreliosis. Immunol Infect Dis 1991;1:271–274.

15 Baran H, Schwarcz R: Regional differences in the ontogenetic pattern of kynurenine aminotransferase in the rat brain. Dev Brain Res 1993;74:283–286.

16 Beal MF, Swartz KJ, Isacson O: Developmental changes in brain kynurenic acid concentrations. Dev Brain Res 1992;68:136–139.

17 Gramsbergen JBP, Schmidt W, Turski WA, Schwarcz R: Age-related changes in kynurenic acid production in rat brain. Brain Res 1992;588:1–5.

18 Moroni F, Russi P, Lombardi G, Beni M, Carla V: Presence of kynurenic acid in the mammalian brain. J Neurochem 1988;51:177–180.

19 Hlinak Z, Krejci I: Kynurenic acid and 5,7-dichlorokynurenic acids improve social and object recognition in male rats. Psychopharmacology 1995;120:463–469.

20 Steele RJ, Stewart MG: 7-Chlorokynurenate, an antagonist of the glycine binding site on the NMDA receptor, inhibits memory formation in day-old chicks *(Gallus domesticus)*. Behav Neural Biol 1993;60:89–92.

21 Baran H, Cairns N, Lubec B, Lubec G: Increased kynurenic acid levels and decreased brain kynurenine aminotransferase I in patients with DOWN syndrome. Life Sci 1996;58:1891–1899.

22 Birch PJ, Grossman CJ, Hayes AG: Kynurenic acid antagonizes responses to NMDA via an action at the strychnine-insensitive glycine receptor. Eur J Pharmacol 1988;154:85–87.

23 Frohman EM, Frohman TC, Dustin ML, Vayuvegula B, Choi B, Gupta A, van den Noort S, Gupta S: The induction of intercellular adhesion molecule 1 (ICAM-1) expression on human fetal astrocytes by interferon-gamma, tumor necrosis factor alpha, lymphotoxin, and interleukin-1: Relevance to intracerebral antigen presentation. J Neuroimmunol 1989;23:117–124.

24 Saito K, Markey SP, Heyes MP: Effects of immune activation on quinolinic acid and neuroactive kynurenines in the mouse. Neuroscience 1992;1:25–39.

25 Morganti-Kossmann MC, Kossmann T, Wahl SM: Cytokines and Neuropathology. TIPS 1992;13:286–291.

26 Benveniste EN: Astrocyte-microglia interactions; in Murphy S (ed): Astrocytes: Pharmacology and Function. San Diego, Academic Press, 1993, pp 355–382.

27 Byrne G, Lehmann LK, Kischbaum JG, Borden EC, Lee CM, Brown RR: Induction of tryptophan degradation in vitro and in vivo: A gamma-interferon stimulated activity. J Interferon Res 1986;6:389–398.

28 Werner ER, Bitterlich G, Fuchs D, Hausen A, Reibneger G, Szabo G, Dierich M, Wachter H: Human macrophages degrade tryptophan upon induction by interferon gamma. Life Sci 1987;42:310–316.

29 Lamelin JP, Vincent C, Fontaine-Legrand C, Revillard JP: Elevation of serum beta 2-microglobulin levels during infections mononucleosis. Clin Immunol Immunopathol 1982;24:55–62.

30 Nilsson K, Evrin PE, Welsh KI: Production of beta 2-microglobulin by normal and malignant human cell lines and peripheral lymphocytes. Transpl Rev 1974;21:53–84.

31 Martinez M, Frank A, Hernanz A: Relationship of interleukin-1 beta and beta 2-microglobulin with neuropeptides in cerebrospinal fluid od patients with dementia of the Alzheimer type. J Neuroimmunol 1993;48:235–240.

32 Tenhunen R, Iivanainen M, Kovanen J: Cerebrospinal fluid beta 2-microglobulin in neurological disorders. Acta Neurol Scand 1978;58:366–373.

33 Brew BJ, Bhalla RB, Fleisher M, Paul M, Khan A, Schwartz MK, Price RW: Cerebrospinal fluid β_2-microglobulin in patients infected with human immunodeficiency virus. Neurology 1989;39:830–834.

34 Tibbling G, Link H, Öhman S: Principles of albumin and IgG analyses in neurological disorders. I. Establishment of reference values. Scand J Clin Lab Invest 1977;37:385–390.

35 Olsson T, Kostulas V, Link H: Improved detection of oligoclonal IgG in cerebrospinal fluid by isoelectric focusing in agarose, double-antibody peroxidase labeling, and avidin-biotin amplification. Clin Chem 1984;30:1246–1249.

36 Shibata K: Fluorimetric microdetermination of kynurenic acid, an endogenous blocker of neurotoxicity, by high performance liquid chromatography. J Chromat 1988;430:376–380.

37 Swartz KJ, Matson WR, MacGarvey U, Ryan EA, Beal MF: Measurement of kynurenic acid in mammalian brain extracts and cerebrospinal fluid by high-performance liquid chromatography with fluorometric and coulometric electrode assay detection. Analyt Biochem 1990;85:363–376.

38 Turski WA, Gramsbergen JBP, Traitler H, Schwarcz R: Rat brain slices produce and liberate kynurenic acid upon expose to *L*-kynurenine. J Neurochem 1989;52:1629–1636.

39 Schmidt W, Guidetti P, Okuno E, Schwarcz R: Characterization of human brain kynurenine aminotransferases using [^{3}H]kynurenine as a substrate. Neuroscience 1993;55:177–184.

40 Heyes MP, Saito K, Crowley JS, Davis LE, Demitrack MA, Der M, Dilling A, Elia J, Kruesi MJP, Lackner A, Larsen SA, Lee K, Leonard HL, Markey SP, Martin A, Milstein S, Mouradian MM, Pranzatelli MR Quearry BJ, Salazar A, Smith M, Strauss SE, Sunderland T, Swedo SW, Tourtellotte WW: Quinolinic acid and kynurenine pathway metabolism in inflammatory and non-inflammatory neurological disease. Brain 1992;115:1249–1273.

41 Rust RS Jr, Dodson WE, Trotter JL: Cerebrospinal fluid IgG in childhood: The establishment of reference value. Ann Neurol 1988;23:406–410.

42 Walker DW, Curtis B, Lacey B, Nitsos I: Kynurenic acid in brain and cerebrospinal fluid of fetal, new born, and adult sheep and effects of placental emboliation. Pediatric Res 1999;45:820–826.

43 Erhardt S, Blennow K, Nordin C, Skogh E, Lindström LH, Engberg G: Kynurenic acid levels are elevated in the cerebrospinal fluid of patients with schizophrenia. Neurosci Lett 2001;313:96–98.

44 Rejdak K, Bartosik-Psujek H, Dobosz B, Kocki T, Grieb P, Giovannoni G, Turski WA, Stelmasiak Z: Decreased level of kynurenic acid in cerebrospinal fluid of relapsing-onset multiple sclerosis patients. Neurosci Lett 2002;331:63–65.

45 Heyliger SO, Goodman CB, Ngong JM, Soliman KFA: The analgesic effects of tryptophan and its metabolites in the rat. Pharmacol Res 1998;38:243–250.

46 Schwieler L, Erhardt S, Erhardt C, Engberg G: Prostaglandine-mediated control of rat brain kynurenic acid synthesis – opposite actions by COX-1 and COX-2 isoforms. J Neural Transm DOI 10.1007/s00702-004-0231-y.

47 Schwarcz R, Baran H, Wu H-Q, Du F, McMaster O: The Neurochemistry of quinolinic acid and kynurenate: Current Concepts; in Meldrum BS, Moroni F, Simon RP, Woods JH (eds): Excitatory Amino Acids. New York, Raven Press, 1991, pp 365–375.

48 Sharfman HE, Goodman JH, Schwarcz R: Eletrophysiological effects of exogenous and endogenous kynurenic acid in the rat brain: in vivo and in vitro. Amino Acids 2000;19:283–297.

49 Fukui S, Schwarcz R, Rapoport SI, Takada Y, Smith QR: Blood-brain barrier transport of kynurenines: Implications for brain synthesis and metabolism. J Neurochem 1991;56:2007–2015.

50 Garton MJ, Keir G, Lakshmi MV, Thompson EJ: Age-related changes in cerebrospinal fluid protein concentrations. J Neurol Sci 1991;104:74–80.

51 Gal EM, Sherman AD: *L*-Kynurenine: Its synthesis and possible regulatory function in brain. Neurochem Res 1980;5:223–239.

52 Wada H, Ito H, Orimo H, Sato A: Kynurenine specifically increases in the cerebrospinal fluid of the aged rats. Biog Amines 1994;3:221–225.

53 Heyes MP, Saito K, Markey SP: Human macrophages convert *L*-tryptophan into the neurotoxin quinolinic acid. Biochem J 1992;283:633–635.

54 Heyes MP, Chen CY, Major EO, Saito K: Different kynurenine pathway enzymes limit quinolinic acid formation by various human cell types. Biochem J 1997;326:351–356.

55 Okuno E, Nakamura M, Schwarcz R: Two kynurenine aminotransferases in human brain. Brain Res 1991;542:307–312.

56 Baran H, Okuno E, Kido R, Schwarcz R: Purification and characterisation of kynurenine aminotransferase I from human brain. J Neurochem 1994;62:730–738.

57 Roberts RC, Du F, McCarthy KE, Okuno E, Schwarcz R: Immunocytochemical localization of kynurenine aminotransferase in the rat striatum: A light and electron microscopic study. J Comp Neurol 1992;326:82–90.

58 Tamburin M, Mostardini M, Benatti L: Kynurenine aminotransferase I (KAT I) isoform gene expression in the rat brain: An in situ hybridization study. Neuroreport 1999;10:61–65.

59 Baran H, Kepplinger B, Kainz A, Draxler M, Ferraz-Leite H, Wallner J, Newcombe J, Erhart H: Kynurenine aminotransferases in human cerebro-spinal fluid. J Neurol 2003;250(suppl 2):II/10, 4.

60 Ferraro TN, Hare TA: Free and conjugated amino acids in human CSF: Influence of age and sex. Brain Res 1985;338:53–60.

61 Sloane JA, Hollander W, Moss MB, Rosene DL, Abraham CR: Increased microglial activation and protein nitration in white matter of the aging monkey. Neurobiol Aging 1999;20:395–405.

62 Laping NJ, Teter B, Nichols NR, Rozovsky I, Finch CE: Glial fibrillary acidic protein: Regulation by hormones, cytokines, and growth factors. Brain Pathol 1994;4:259–275.

63 Lee G, Dallas S, Hong M, Bendayan R: Drug transporters in the central nervous system: Brain barriers and brain parenchyma considerations. Pharmacol Rev 2001;53:569–596.

64 Münch G, Schinzel R, Loske C, Wong A, Durany N, Li JJ, Vlassara H, Smith MA, Perry G, Riederer P: Alzheimer's disease – synergistic effects of glucose deficit, oxydative stress and advanced glycation endproducts. J Neural Transm 1998;105:439–461.

65 Morgan TE, Rozovsky I, Golgsmith SK, Stone DJ, Yoshida T, Finch CE: Increased transcription of the astrocyte gene GFAP during middle-age is attenuated by food restriction: Implications for the role of oxidative stress. Free Radic Biol Med 1997;23:524–528.

66 Magnusson KR: Aging of glutamate receptors: Correlation between binding and spatial memory performance in mice. Mech Ageing Dev 1998;104:227–248.

67 Magnusson KR, Cotman CW: Effects of aging on NMDA and MK801 binding sites in mice. Brain Res 1993;604:334–337.

68 Kumoro H, Rakic P: Modulation of neuronal migration by NMDA receptors. Science 1993;260:95–97.