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Kynurenine metabolism in Alzheimer's disease

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Summary. L-kynurenine (L-KYN) serves as a substrate for the synthesis of neurotoxic 3-OH-kynurenine (3-OH-KYN) and neuroprotective kynurenic acid (KYNA). KYNA is able to interact with ionotropic excitatory amino acid receptors that are involved in a variety of neurodegenerative disorders. The purpose of the present study was to investigate the biosynthetic machinery of KYNA in several regions of Alzheimer's disease (AD) brain. The endogenous levels of L-KYN, 3-OH-KYN and KYNA in frontal cortex, caudate nucleus, putamen, hippocampus, and cerebellum of 11 autopsy confirmed cases of AD and 13 age-matched controls were analyzed. Subsequently, the activity of two proteins responsible for the production of KYNA, kynurenine aminotransferases I and II (KAT I and KAT II), was investigated. There was a trend for a decrease of L-KYN and 3-OH-KYN in all examined regions of AD brain, as compared to controls. However, KYNA was increased significantly in the putamen and caudate nucleus of AD, by 192 and 177%, respectively. In other areas of AD brain only a minor increase of KYNA was present. Elevated KYNA in the caudate nucleus and putamen correlated with a significant increase of KAT I activities in both nuclei – 157 and 147%, respectively. A minor increase of KAT II was measured only in the caudate nucleus of AD subjects. Kinetic analysis of KAT I and II performed in the caudate nucleus of AD patients revealed a marked increase of V_{max}, by 207 and 274% of controls, respectively. K_m value for L-KYN using pyruvate as amino acceptor was significantly higher for KAT II (247% of controls). The present data indicate an elevated kynurenine metabolism in AD brain. A marked increase of KYNA in the caudate nucleus and putamen may compensate the hyperactivity of the striato-frontal loop in AD brains. Blockade of NMDA receptors by KYNA may be responsible for impaired memory, learning and cognition in AD patients.

Keywords: Alzheimer's disease, kynurenine, kynurenine aminotransferase, kynurenic acid, biosynthesis.

Introduction

Dementia is a major health problem in the elderly, especially in those aged 65 years and over. Alzheimer's disease (AD) is the most common cause of dementia. Patients with AD present with progressive impairment of memory and cognition (McKhann et al., 1984). The disease selectively affects certain neuronal populations including basal forebrain cholinergic neurons, glutamatergic neurons in hippocampus and cortex and, less, brainstem monoaminergic neurons (Davies, 1979; Hyman et al., 1984, 1987; Mann and Yates, 1986) as well as cortical synapses (Lassmann et al., 1992; Terry et al., 1991; Heffernan et al., 1998). A larger number of senile plaques, neurofibrillary tangles and more severe neuronal and synapse loss have been found in AD patients compared to nondemented individuals of the same age (Tomlinson and Corsellis, 1984; Terry et al., 1991; Hof et al., 1995; Jellinger, 1998).

One of the metabolic pathways of particular biologic interest in tryptophan catabolism is that leading to niacin synthesis, i.e. the kynurenine pathway (see Fig. 1), (Brown, 1971). This pathway has also another biologically notable function, i.e. the synthesis of two neuroactive metabolites, quinolinic acid (QUIN) with excitatory, and kynurenic acid (KYNA) with inhibitory properties (Stone, 1993). Along the kynurenine pathway L-kynurenine (L-KYN) is catabolized by kynurenine-3-hydroxylase, followed by kynureninase and 3-hydroxyanthranilic acid oxygenase to QUIN. QUIN is catabolized to nicotinic acid mononucleotide via quinolinic acid phosphoribosytransferase (QPRT) and, finally to active coenzyme nicotinamide adenine dinucleotide (NAD). Whereas, KYNA is synthesized by irreversible transamination of L-KYN (Gal and Sherman, 1978), in mammalian peripheral organs, several aminotransferases are able to convert L-KYN to KYNA. In human brain, two kynurenine aminotransferases I and II (KAT I and KAT II) are responsible for the production of its bioprecursor L-KYN (Okuno et al., 1991a) (Fig. 1). These two enzymes, KAT I and KAT II, have been characterized and show distinct catalytic characteristics (Okuno et al., 1991a; Schmidt et al., 1993; Baran et al., 1994). However, the cell type expressing KAT I and KAT II is not known yet.

A large body of data from experimental animals has indirectly implicated this two metabolites of the kynurenine pathway, QUIN and KYNA in the pathogenesis of chronic neurodegenerative disorders (Foster et al., 1984; Schwarcz and Price, 1991; McMaster et al., 1991, 1993; Du et al., 1998). Particular interest has been focused on AD because of the similarities between QUIN-induced lesions in rats (Schwarcz and Köhler, 1983) and post mortem findings in human AD brain (Davies, 1979). Studies of CSF and biopsy samples of AD brain revealed, however, no changes of QUIN concentration (Moroni et al., 1986; Mouradian et al., 1989).

Activation of N-methyl-D-aspartate (NMDA, a glutamate agonist) receptors appears to be an important factor in the establishment of long-term potentiation (LTP) (Bliss and Collingridge, 1993). Overstimulation of these receptors may lead to structural breakdown of nerve cells and is likely to be

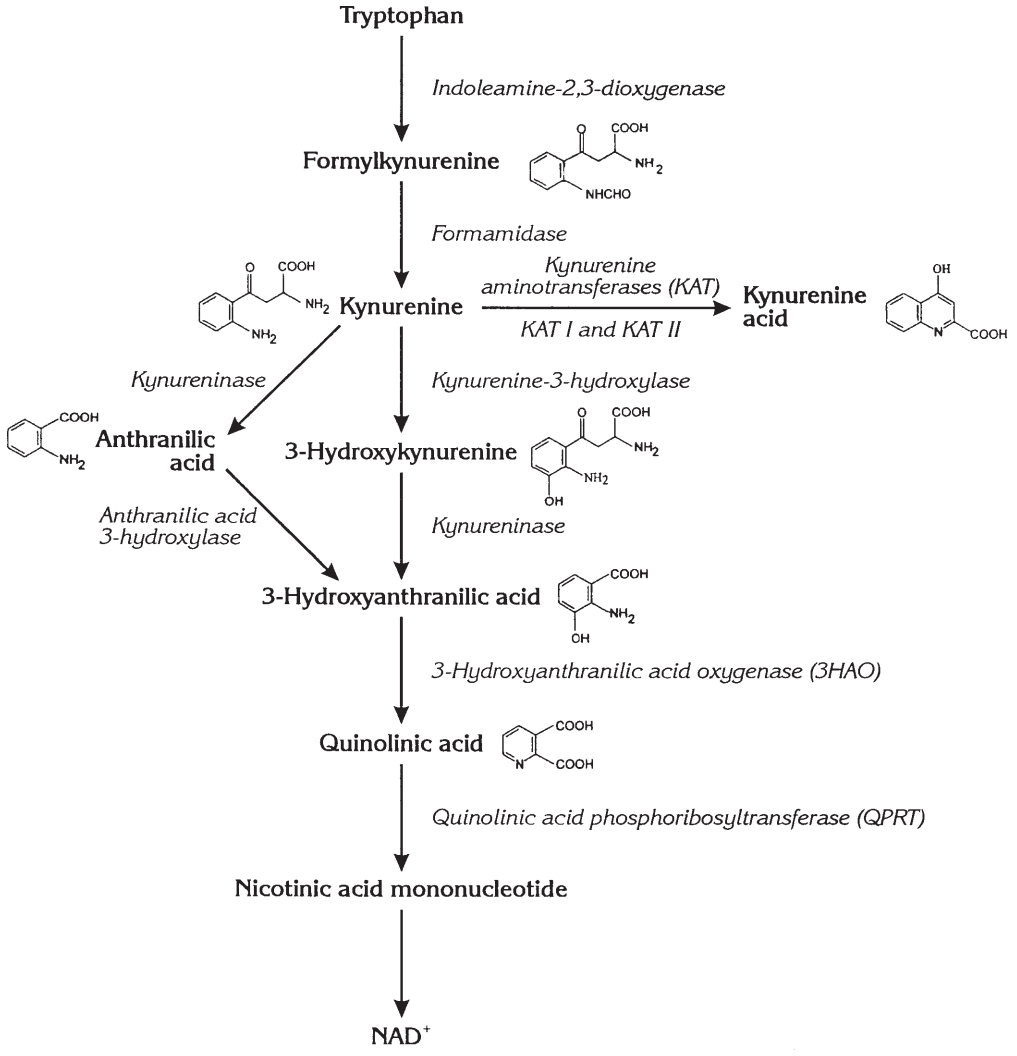

Fig. 1. The kynurenine pathway of tryptophan degradation

involved in the pathogenesis of several human neurodegenerative disorders e.g. Huntington's disease, hypoxia-ischemia or epilepsy (Schwarcz and Price, 1991; Schwarcz, 1992; Stone, 1993).

KYNA modulates the NMDA receptors (Stone, 1993), has neuroprotective and anticonvulsive activities (Foster et al., 1984); it is synthesized by enzymic conversion of L-KYN to KYNA (Gal and Sherman, 1978). Dysfunction of KYNA synthesis in the brain has been hypothesized as an important factor contributing to neuronal degeneration (Foster et al., 1984). In vivo studies have shown that reduction of KYNA synthesis in the rat brain using non-specific inhibitors can lead to neurotoxic action (Beal et al., 1991; McMaster et al., 1991, 1993; Fu et al., 1998). Decreased KYNA production has been demonstrated in the brain of Huntington's patients (Beal et al., 1990; Jauch et al., 1995) which present neuropathological features similar to those

produced by non-specifc inhibitor in experimental animals (Beal et al., 1991). However, regardless of the excitotoxic action of QUIN or GABA, an impairment of intracellular energy metabolism should be considered, too (Beal et al., 1991; McMaster et al., 1991, 1993). Preferential loss of layer III of entorhinal cortex after local injection of a non-specific inhibitor of KYNA synthesis was suggested as a significant factor in the pathophysiology of temporal lobe epilepsy (Du et al., 1998), and likely can be involved in the pathophysiology of several neuropsychiatric diseases, such as AD and schizophrenia (Hyman et al., 1986; Arnold et al., 1991).

Studies in experimental animals and in neuronal hybrid cells have shown that 3-OH-KYN, a metabolite of the kynurenine-pathway, may cause seizures and neurotoxic action (Lapin, 1981; Eastman and Guilarte, 1989). Person and Reynolds (1992) demonstrated elevated 3-OH-KYN levels in the putamen, temporal and frontal cortices of Huntington's patients. They suggested that these changes could be of aetiological significance. A preliminary study of 3-OH-KYN in AD indicated only a trend for increased 3-OH-KYN (Person and Reynolds, 1992).

The aim of present study was to investigate the metabolism of L-KYN in the brain of AD patients. We measured the content of endogenous L-KYN, 3-OH-KYN, and KYNA in the frontal cortex, hippocampus, caudate nucleus, putamen, and cerebellum of AD patients and age-matched non-demented controls. We measured also the activities of KYNA synthesizing enzymes KAT I and KAT II. Subsequently, we characterized the kinetic parameters of KAT I and KAT II in the striatum of AD patients and age-matched non-demented controls.

Material and methods

Chemicals

L-KYN, 3-OH-KYN, KYNA, pyruvate, pyridoxal-5′-phospate and 2-amino-2-methyl-1-propanol (AMP), were purchased from Sigma. All other chemicals used were of the highest commercially available purity.

Brain tissue

Postmortem human brain samples of frontal cortex, hippocampus, putamen, caudate nucleus, and cerebellum were obtained from 11 autopsy confirmed patients with definite, i.e. severe AD (NIA and CERAD criteria) (Mirra et al., 1991) aged 81.0 ± 1.9 years and 13 non-demented controls aged 80.1 ± 2.4 years. Ratio male/female was 2/9 for AD patients and 7/6 for controls. Postmortem time was 30.4 ± 3.6 (hrs) in AD and 29.7 ± 3.9 (hrs) in controls. Brain samples were stored at −70°C before analysis.

In addition, brain samples of single cases of vascular encephalopathy, Parkinson's disease, and cerebral infarction, and serum and cerebrospinal fluid (CSF) from single patients with probable AD and controls were analysed.

Tissue preparation

Brain samples from each subject were homogenized by a Potter followed by a Polytron (Kinematics GmbH, Luzern, Switzerland) in an ice bath in 6 volumes (wt/vol) of 5 mM Tris-acetate buffer pH 7.4 containing 50 μM pyridoxal-5′-phosphate and 10 mM

mercaptoethanol. Obtained homogenate was divided in two parts one for L-KYN, 3-OH-KYN and KYNA determination and the other for KATs activities measurements.

Measurement of L-KYN and 3-OH-KYN and KYNA

Homogenate was mixed with 0.2 M HCl (vol/vol) and centrifuged (20 min, 14,000 rpm). The supernatant was applied to a Dowex 50 W cation exchange column prewashed with 0.1 M HCl. Subsequently, the column was washed with 1 ml 0.1 M HCl and 1 ml distilled water, and KYNA was eluted with 2 ml distilled water (Turski et al., 1988) and L-KYN and 3-OH-KYN was eluted with 2 ml of 1 M NH_4OH (Nakamura et al., 1986). KYNA was quantitated by high performance liquid chromatography (HPLC) coupled with fluorescence detection as introduced by Swartz et al. (1990). The HPLC system used for analysis of KYNA consisted of the following: pump (Shimadzu, LC-6A), Fluorescence Detector (Shimadzu, RF-535) set at an excitation wavelenght of 340 nm and an emission wavelength of 398 nm, and a Shimadzu C-R5A Chromatopac Integrator. The mobile phase (isocratic system) consisted of 50 mM sodium acetate, 250 mM zinc acetate and 4% acetonitrile, pH 6.2, and was pumped through a 10 cm $\times$ 0.4 cm column (HR-80, C-18, particle size 3 μM, InChrom, Austria) at a flow rate of 1.0 ml/min, run at room temperature (23°C). L-KYN and 3-OH-KYN was quantitated by HPLC coupled with UV detector at 365 nm. Mobile phases contained 0.1 M ammonium acetate, 0.1 M acetic acid, and 2% acetonitrile (Chiarugi et al., 1995).

Determination of KAT I and KAT II activities

The activities of the KATs were assayed by the method of Mason (1954) as modified by Okuno et al. (1991a) and Baran et al. (1995). Briefly, the reaction mixture contained the homogenate, 2 μM L-KYN, 1 mM pyruvate, 70 μM pyridoxal 5′-phosphate and 150 mM AMP buffer, pH 9.6 (for KAT I) or 150 mM Tris-acetate buffer, pH 7.4 (for KAT II), in a total volume of 0.2 ml. After the incubation for 16 hrs at 37°C (linearity of enzyme activity up to 16 h was ascertained in pilot experiments) the reaction was terminated by the addition of 10 μl of 50% TCA. Subsequently, 1 ml of 0.1 M HCL was added and denatured protein was removed by 5 min centrifugation (Eppendorf Microfuge). The supernatant was applied to a Dowex 50 W cation exchange column. Eluted KYNA from the column was quantitated by HPLC method, as described above. The blanks were obtained by using tissue which has been heat inactivated for 30 min in a boiling water bath.

Kinetic analysis

The kinetics of KAT I and KAT II were assessed by varying the concentration of L-KYN. The substrate concentration ranged from 150 μM to 10 mM for KAT I and from 150 μM to 25 mM for KAT II (with pyruvate concentration fixed at 1 mM). Apparent K_m values were calculated from double-reciprocal plots.

Protein determination

Protein was measured according to the method of Bradford (1976) using a commercially available kit (BIO-RAD) and bovine serum albumin as a standard.

Statistics

All data are given as means $\pm$ S.E.M. For comparison of groups the Mann Whitney U-Test was applied. The levels for statistical significance was taken as $p < 0.05$.

Results

The concentrations of KYN, 3-OH-KYN and KYNA measured in control subjects were in close aggreement with those of previous studies (Joseph, 1978; Gal and Shermaqn, 1980; Heyes, 1988; Beal et al., 1990; Jauch et al., 1994; Baran et al., 1996). The highest levels of L-KYN were measured in the cerebellum (18.9 pmol/mg protein) and putamen (17.1 pmol/mg protein). The lowest L-KYN was found in hippocampus (11.9 pmol/mg protein) (Table 1). The concentration of 3-OH-KYN did not vary among different brain regions and all were in the low pmol/mg protein concentration. There was only a moderate variation of KYNA levels within the CNS with less then a 4 fold difference between putamen (6.42 pmol/mg protein) and cerebellum (1.63 pmol/mg protein), while caudate nucleus, frontal cortex, and hippocampus had less then 1.5, 1.7, and 2.2 fold, respectively, of the concentration present in putamen (Table 1).

There was a mild but not significant reduction of L-KYN and 3-OH-KYN in all examined regions of AD brain, whereas the endogenous kynurenine metabolite KYNA showed a considerable increase. The most severe and significant elevation of KYNA was seen in the putamen and caudate nucleus by 191.9 and 176.9%, respectively, while the other brain areas, hippocampus, cerebellum, and frontal cortex, showed only a moderate and non-significant elevation of KYNA (Table 1).

A marked increase of KYNA in the caudate nucleus and putamen was in line with a significant elevation of KAT I activity by 156.6 and 147.3% of controls, respectively (Table 2). KAT II was enhanced moderately and only in the caudate nucleus (Table 2). The kinetic characteristics of KATs examined by varying concentration of L-KYN revealed a normal K_m value for KAT I and nearly 2.5 fold higher K_m value for KAT II in the caudate nucleus of AD brain, as compared to controls (Table 3). Moreover, we found a significant differences in V_{max} values for KAT I and KAT II (207% and 247% of control, $p < 0.01$) in the caudate nucleus of AD patients, as compared to controls (Table 3). The shift in K_m value of KAT II may be due to a decreased enzyme affinity (inhibition by endogenous unknown substance(s)), while increaced V_{max} may result from alteration (increase) of enzyme expression. Moreover, we found significant differences in V_{max} values for KAT I and II (207% and 247% of control, $p > 0.01$) in the caudate nucleus of AD patients.

Additional studies in single instances of other brain disorders showed somewhat different results: while L-KYN was mildly to considerably reduced and 3-OH-KYN showed no considerable changes in most examined brain areas of vascular encephalopathy and Parkinson's disease, KYNA was considerably increased in both disorders, particularly in the putamen and caudate nucleus (Table 4). While KAT I was mildly reduced in vascular encephalopathy, it was mildly increased in the hippocampus and in caudate nucleus of one Parkinsonian brain. On the other hand, KAT II was only increased in the frontal cortex of vascular encephalopathy, while the other brain areas showed inconsistent results (Table 4). Further studies are needed to confirm these very preliminary data in non-AD brain disorders.

Table 1. Kynurenine metabolites in human brain of Alzheimer's patients and controls

Brain region	Group	L-Kynurenine		3-OH Kynurenine		Kynurenic acid	
		(pmol /mg protein)	% of control	(pmol/mg protein)	% of control	(pmol/mg protein)	% of control
Frontal cortex	CO	15.3 ± 2.7		5.03 ± 0.80		3.88 ± 0.95	
	AD	12.9 ± 1.3	79.5	4.10 ± 0.53	81.5	4.79 ± 0.85	123.4
Hippocampus	CO	11.9 ± 1.8		5.27 ± 0.75		2.88 ± 0.56	
	AD	11.1 ± 1.7	92.9	4.30 ± 0.51	81.7	3.88 ± 0.84	134.7
Putamen	CO	17.1 ± 2.6		6.26 ± 0.79		6.42 ± 1.49	
	AD	14.5 ± 1.6	84.7	4.79 ± 0.51	76.4	12.32 ± 2.08	191.9*
Nucleus Caudate	CO	16.2 ± 3.4		5.83 ± 0.90		4.34 ± 1.06	
	AD	12.3 ± 1.8	75.6	4.05 ± 0.52	69.6	7.68 ± 1.65	176.9*
Cerebellum	CO	18.9 ± 3.7		5.67 ± 0.64		1.63 ± 0.37	
	AD	15.8 ± 2.4	83.0	4.83 ± 0.48	85.3	2.32 ± 0.61	142.3

Data represent a mean ± SEM of 13, 14 controls and 10, 11 AD samples. Significances: *$p < 0.05$

Table 2. Kynurenine aminotransferases (KATs) in different brain regions in Alzheimer patients and controls

	Kynurenine aminotransferases (KATs) (pmol/mg protein/h)					
	KAT I			KAT II		
Region	Control	Alzheimer	% of control	Control	Alzheimer	% of control
Frontal cortex	0.47 ± 0.07	0.47 ± 0.08	100.0	0.61 ± 0.07	0.66 ± 0.08	108.2
Hippocampus	0.33 ± 0.04	0.33 ± 0.04	100.0	0.42 ± 0.07	0.43 ± 0.03	102.4
Putamen	0.55 ± 0.08	0.81 ± 0.14	147.3*	0.54 ± 0.08	0.61 ± 0.09	112.9
Caudate Nucleus	0.53 ± 0.09	0.83 ± 0.10	156.6*	0.57 ± 0.09	0.77 ± 0.08	135.1
Cerebellum	0.31 ± 0.06	0.38 ± 0.07	122.6	0.38 ± 0.06	0.44 ± 0.06	115.8

Data represent a mean ± SEM of 12 ÷ 13 controls and 10 ÷ 11 Alzheimer's patients. Significances: $*p < 0.05$

Examination of serum and CSF in a small number of AD patients showed a significant increase of KYNA in both serum and CSF (Table 5). These data also need confirmation in a larger number of patients.

Discussion

It is well known that excitatory amino acids (EAAs) acting through specific receptors can cause neuronal dysfunction and subsequent nerve cell death. These receptors are critically involved in the LTP, an experimental model of learning and memory (Muller et al., 1989). LTP has been demonstrated in certain synaptic pathways of hippocampus which are glutamatergic and contain high densities of NMDA receptors (Stone, 1993). Blockade of NMDA receptors by the competative antagonist AP5 resulted in impairment of several tasks in rats (Morris et al., 1986; Danysz et al., 1988). KYNA, a broad-spectrum antagonist of NMDA receptors, has a particularly high affinity for the strychnine insensitive glycine site (Stone, 1993) and shows anticonvulsive and neuroprotective activities (Foster et al., 1984). Increased levels of KYNA in rat brain were reported in conditions associated with cognitive impairment including aging (Moroni et al., 1988; Gramsbergen et al., 1992).

Table 3. Kinetic characteristics of KAT I and KAT II in caudate nucleus of Alzheimer (AD) patients and controls

	KAT I		KAT II	
	Km (mM)	Vmax (nmol/mg protein/h)	Km (mM)	Vmax (nmol/mg protein/h)
Control	5.21 ± 0.81	1.49 ± 0.04	10.56 ± 2.73	6.02 ± 1.21
Alzheimer	4.25 ± 0.14	3.08 ± 0.21*	26.09 ± 4.19*	16.50 ± 3.64*

Data are the mean ± SEM of 3 separate kinetics. Significances: $*p < 0.01$

Table 4. Concentration of L-KYN, 3-OH-KYN and KYNA and enzymes activities of KAT I and KAT II in some neurodegenerative disorders

	Brain region	Controls	VENC	PD	INF
L-KYN	FrCTX	15.3 ± 2.7	10.72 ± 2.42	11.87	14.25
	HIPP	11.9 ± 1.8	13.26 ± 0.41	13.40	14.24
	PUT	17.1 ± 2.6	10.94 ± 1.95	9.11	21.50
	CN	16.2 ± 3.4	12.55 ± 0.56	10.24	18.43
	CER	18.9 ± 3.7	9.97 ± 2.20	8.50	19.92
3-OH-KYN	FrCTX	5.03 ± 0.80	7.09 ± 0.43	5.86	8.20
	HIPP	5.27 ± 0.75	9.02 ± 0.70	8.05	8.80
	PUT	6.26 ± 0.79	7.48 ± 0.75	6.15	8.78
	CN	5.83 ± 0.90	8.10 ± 0.21	5.90	7.82
	CER	5.67 ± 0.64	7.19 ± 0.03	7.57	9.13
KYNA	FrCTX	3.88 ± 0.95	6.24 ± 2.89	5.78	6.26
	HIPP	2.88 ± 0.56	4.53 ± 0.62	7.74	8.04
	PUT	6.42 ± 1.49	14.30 ± 2.23	10.13	11.79
	CN	4.34 ± 1.05	10.59 ± 0.89	9.44	10.83
	CER	1.63 ± 0.37	1.81 ± 0.71	1.89	3.64
KAT I	FrCTX	0.47 ± 0.07	0.42 ± 0.01	0.35	1.34
	HIPP	0.33 ± 0.04	0.39 ± 0.18	0.63	1.08
	PUT	0.55 ± 0.08	0.30 ± 0.09	0.58	1.08
	CN	0.53 ± 0.09	0.39 ± 0.01	0.70	1.22
	CER	0.31 ± 0.06	0.22 ± 0.02	0.46	0.44
KAT II	FrCTX	0.61 ± 0.07	1.00 ± 0.12	0.53	1.39
	HIPP	0.42 ± 0.07	0.48 ± 0.16	0.62	1.22
	PUT	0.54 ± 0.08	0.63 ± 0.27	0.34	0.83
	CN	0.57 ± 0.09	0.37 ± 0.16	0.84	1.34
	CER	0.38 ± 0.06	0.54 ± 0.19	0.54	0.44

L-KYN, 3-OH-KYN and KYNA are expressed in pmol/mg Protein; KAT I and KAT II are expressed in pmol/mg protein /h; Control data represent a mean ± SEM of n = 10, 11; VENC (n = 2); PD (n = 1); INS (n = 1)

VENC Vascular Encephalopathy; *PD* Parkinson Disease; *INF* Cerebral Infarct; *L-KYN* L-Kynurenine; *3-OH-KYN* 3-OH-Kynurenine; *KYNA* Kynurenic Acid; *KAT I and II* Kynurenine Aminotransferase I and II; *Fr CTX* Frontal Cortex; *HIPP* Hippocampus; *PUT* Putamen; *CN* Caudate Nucleus; *CER* Cerebellum

Table 5. KYNA concentration in CSF and serum in AD and control patients (n)

Group	KYNA in serum (fmol/μl)	KYNA in CSF (fmol/μl)
Controls (72.3 ± 3.9 yrs); MMS 28.7 ± 2.1	3.53 ± 0.94 (4)	0.49 ± 0.04 (5)
AD (73.2 yrs); MMS 16/30	26.09 (1)	3.68 ± 0.57 (2)

MMS Mini Mental state

Studies on KYNA metabolism in a variety of neurodegenerative disorders revealed an involvement of endogenous NMDA antagonists in the brain tissue. Thus, a reduction of L-KYN and KYNA content was found in the frontal cortex and putamen of Parkinson's disease (PD) patients (Ogawa et al., 1992), while one PD brain in our study revealed decreased L-KYN but increased KYNA, particularly in the striatum. A reduction of KYNA in the caudate nucleus and lowered KAT I and KAT II activities in the putamen were demonstrated in Huntigton's disease (HD) (Beal et al., 1990; Jauch et al., 1995). Increased KYNA levels and reduced KAT I were seen in the frontal and temporal cortices in Down's syndrome (DS) (Baran et al., 1996). Depletion of KYNA levels has been related to movement disorders e.g. in HD, while the increase of KYNA content may have importence for mental disabilities seen in AD, PD, and DS. A link between a hyperfunctional KYNA system and occurrence of psychotic phenomena has been proposed in schizophrenia (Schwarcz et al., 1990). In this respect, quantitative analysis of the cerebral cortex revealed similar distribution patterns of pathological lesions (affected layer II of the enthorhinal cortex followed by the hippocampus and neocortex) in DS, schizophrenia, and AD (Hyman et al., 1987; Arnold et al., 1991; Braak and Braak, 1991; Hof et al., 1995; Jellinger, 1998).

No information about the cellular localization of human brain KAT I and II has been described so far and the sources of reported enzyme activities remain speculative. Preliminary work on the cellular localization of KAT I and KAT II using excitotoxin-lesioned rat striatum suggested cerebral KYNA synthesis by KAT II in non-neuronal cells in gliotic striatum (Guidetti et al., 1997). This is in line with previous immunohistochemical studies in rat hippocampus and striatum showing glial localization of KAT II protein (named kynurenine-pyruvate aminotransferase) (Okuno et al., 1990; Du et al., 1992; Roberts et al., 1992). A marked cerebellar KYNA increase in response to systemically applied neurotoxin kainic acid (Baran et al., 1995) and a prominent role of KAT I in naive cerebellum (a ratio KAT I/KAT II = 55) suggest that cerebellar KYNA synthesis occurs in non-gliotic (neuronal) cells preferentially containing KAT I (Guidetti et al., 1997). It should be mentioned that the cerebellum is not known to be particularly sensitive to systemic application of the neurotoxins, i.e. induction of neurodegeneration and accompanying glial proliferation (Schwarcz and Köhler, 1983). AD brains are characterized by an increase in number of cortical astrocytes (Beach et al., 1989), however, this pathological condition poorly correlates with the changes of kynurenine metabolism. On the other hand, whether an elevation of KYNA synthesis could be related to microglial and /or astroglial actication in the cascade of pathologic processes in AD (Münch et al., 1998) needs further elucidation.

Different enzyme characteristics of human KAT I and KAT II, i.e. pH optimum, substrate specificity and the effect of L-amino acids (Okuno et al., 1991a; Schmidt et al., 1993; Baran et al., 1994), suggest that the activities of both proteins can be influenced by fluctuations in the intracellular concentration of endogenous substrates, which may frequently exhibit significant preference for one and/or the other enzyme.

Glutamatergic innervation of the hippocampus and NMDA receptors present on these neurons are involved in the regulation of many brain functions including memory (Collingridge and Bliss, 1987; McGaugh, 1989). A significant reduction of glutamate in hippocampus and cortex has been shown in AD (Arai, 1984; Ellison, 1986; Hardy et al., 1987; Hyman et al., 1987). Furthermore, Procter et al. (1989) demonstrated that a strychnine-insensitive glycine recognition site of NMDA receptor involved in glutamatergic neurotransmission is altered selectively in AD. Elevated KYNA, reduced glutamate function and fluctuation at the glycine site of the NMDA receptor may significantly influence processes leading to impaired learning and memory capacity.

A recent study by Pittaluga et al. (1997) has suggested that glycine at the NMDA receptor can play a dual action, the well known allosteric modulation of the NMDA recognition site (Johnson and Ascher, 1987) or a protective action against KYNA following binding to sites different than the strychnine-insensitive one. This idea is supported by behavioural animal studies with putative cognition enhancers. For example, the NMDA glycine site ligand D-cycloserin was shown to have a positive influence on memory consolidation, retrieval and learning processes (Monahan et al., 1989). Recently, it has been reported to block epileptic events (Baran et al., 1994a). D-cycloserin is able to reverse KYNA antagonism at NMDA receptors (Pittaluga et al., 1995). These data suggest that the NMDA receptor agonists can modulate additional processes involved in memory and learning. Interaction and/or relationships which probably exist between different binding sites on the NMDA receptor and specific conditions (compounds, age) may result in differential behavioural effects and memory performances. In view of the importence of glutamate receptors, particularly those of the NMDA type, KYNA has been reported to improve short memory (Hlinak and Krejci,1995), but also may influence conditions associated with cognitive deficits and aging (Moroni et al., 1988; Heyes et al., 1990; Gramsbergen et al., 1992).

Studies on the effect of aging on blood-brain barier (BBB) revealed a decreased ability of tryptophan transport (Tang and Melethil, 1995). However, the concentration of tryptophan in brain of AD patients was not significantly different from controls (Arai et al., 1984; Storga et al., 1996). L-KYN, a common substrate for KYNA and 3-OH-KYN synthesis, is readily penetrating the BBB (Gal and Scherman, 1978; Fukui et al., 1991) and has a bearing on the disposition of both metabolites. Although, the BBB is known to be compromised in AD (Skong et al., 1998) raising the possibility of inhanced penetration by kynurenines, we observed only a tendency for decreased L-KYN content in AD brain. Similar data were demostrated by Heyes et al. (1992) in CSF, while no change of L-KYN in CSF of AD patients was reported by Tohgi et al. (1995). Determination of 3-OH-KYN revealed a trend of decreasing 3-OH-KYN content in AD brain as compared. A moderate change of 3-OH-KYN was also reported by Pearson and Reynolds (1992), while Tohgi et al. (1995) described a significant decrease of 3-OH-KYN in CSF of AD patients. Accumulated data neither indicate an

increase of neurotoxic 3-OH-KYN nor suggest directly its aetiological importance in AD.

Elevated brain KYNA levels in our AD sample correlated with preliminary data of increased KYNA content in both CSF and plasma (Table 5), while Heyes et al. (1992) reported decreased (ca. 30%) KYNA content in CSF of AD patients. This discrepancy is due to a marked difference between endogenous control levels presented by Heyes et al. (1992) (3.49 nM) and our measurement (0.49 nM), while in AD patients the CSF content of KYNA was comparable. Increased plasma KYNA cannot be explained by an efflux of KYNA from brain tissue but suggests the involvement of an additional pathway from indole-3-pyruvic acid (Moroni et al., 1991; Politi et al., 1991) and/or an interference with degradation of KYNA metabolism to quinaldic acid (Takahashi et al., 1956). Substantial KYNA formation from indole-3-pyruvic acid has been found in the presence of free radical forming systems (Politi et al., 1991). Therefore, the increase of KYNA in selected AD brain areas can be related to oxidative stress recently discussed as an important factor in the pathogenesis of AD (Münch et al., 1998). KYNA produced from L-KYN is rapidly removed from the brain via a probenecid-sensitive mechanism (Moroni et al., 1988). Administration of probenecid or L-KYN increases significantly the levels of KYNA in the rats brain (Moroni et al., 1988; Vecsei et al., 1992b). Elevated KYNA content in CSF and plasma was reported in AD patients after probenecid and L-KYN administration, too (Vecsei et al., 1992a), but unfortunately, without any comments on the cognitive function of treated AD patients. In our AD sample none of the patients had received these substances prior to death.

The selective elevation of KYNA biosynthesis in the caudate nucleus of AD patients may be due to compensatory hyperactivity of the striato-frontal loop due to neuronal loss in cortical target areas. As KYNA antagonizes the NMDA receptors involved in cognitive functions, changes in KYNA metabolism may represent a pathogenic factor in neuronal dysfunction causing cognitive deterioration in AD. The negative aspects of KYNA elevations should be considered in therapeutic strategies by using drugs which increase the putative cognition and the neuroprotective potential of KYNA acting at other glutamate receptors. The NMDA agonist D-cycloserin has been shown to facilitate activation of NMDA receptors in brain membranes of AD (Chessell et al., 1991) and to improve learning in a human model of cognitive deficits associated with dementia and ageing (Wesnes et al., 1991) and as well has been suggested to have a therapeutic action by schizophrenic patients (Cascella et al., 1994).

Conclusions

1. The present data do not indicate a deficiency of KYNA metabolism in the brain of AD patients. 2. By contrast, elevated KYNA in caudate nucleus and putamen and a trend toward increased KYNA in other areas indicate elevation of KYNA synthesis in AD brain. 3. KAT I was increased in caudate nucleus and putamen, while KAT II activity was enhanced moderately and

only in caudate nucleus. No alteration of KAT I and KAT II activities were seen in frontal cortex, hippocampus and cerebellum of AD brain. 4. Kinetic analyses, performed in caudate nucleus of AD patients, showed an approximately twofold increase in K_m value for KAT II. V_{max} value was increased approximately 2.5-fold for both KAT I and KAT II, likely due to presence of either endogenous activators and/or increased amount of proteins. 5. Elevated brain KYNA levels in a small sample of AD patients correlated well with increased KYNA content in both CSF and plasma of these patients. 6. Studies in single instances of vascular encephalopathy and Parkinson's disease revealed inconsistent results that need to be confirmed by further studies.

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