ORIGINAL RESEARCH

Endogenous Kynurenine Aminotransferases Inhibitor is Proposed to Act as "Glia Depressing Factor" (GDF)

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Abstract: The endogenous neuroinhibitory amino acid receptor antagonist kynurenic acid (KYNA) has been hypothetically linked to physiological processes and to the pathogenesis of several brain disorders. The aim of this study was to search KYNA metabolism i.e. KYNA levels and enzymes synthesising KYNA kynurenine aminotransferase I and II (KAT I and II) in the central nervous system (CNS) and in the peripheral nervous system. Within the investigated species we found a remarkably low KYNA content (3.4 nM) in piglet's serum compared to rat and human serum. Furthermore, in contrast to high KAT activity present in rat and human livers, a lack of KAT I and KAT II activity was found in piglet liver and other piglet peripheral organs. Therefore we attempted to find a reason for the absence of KYNA formation in piglet peripheral tissue and we researched to find if KYNA formation in rat liver homogenate (measured under standard assay conditions for KAT activity) can be influenced by the application of piglet tissue homogenates and other body fluids. KYNA formation in rat liver homogenate was investigated in the presence of piglet liver, piglet brain, rat brain and human brain homogenates, and also in the presence of cerebrospinal fluid (CSF) of the control and of Multiple Sclerosis patients. We found a significant and dose dependent reduction of rat liver KAT I and KAT II activities in the presence of piglet brain, piglet liver, and human brain, but not in the presence of rat brain homogenate. Interestingly, CSF of the human control subjects significantly lowered rat liver KAT I activity. Furthermore, the inhibitory effect of CSF of Multiple Sclerosis (MS) patients was significantly weaker when compared to the CSF of control subjects. Our data, for the first time, indicated the presence of active component(s)—depressing factor—in the body, which was able to block KYNA formation. Reduced KAT inhibitory effect by CSF of MS patients would suggest a lowered "depressing factor" level in CSF of MS patients and is possibly responsible for an enhancement of KYNA formation and for glia activation and gliosis in the CNS. Subsequently, two fractions obtained after centrifugation of CSF from patients with Neuroborreliosis showed a significantly different ability to block KAT I activity. The CSF-sediment fraction exerts a stronger inhibitory activity than the CSF-supernatant fraction, supporting further the presence of a depressing factor. For the first time, data revealed and demonstrated the ability of endogenous components to block KYNA's synthesis. We propose that a glia depressing factor (GDF), which is abundantly present in the body, might simultaneously control glia cell's KAT activity, respectively KYNA synthesis and also glia proliferation. The mechanism(s) of action, the composition and structure of this factor needs to be further elaborated.

Keywords: kynurenine aminotransferases, kynurenic acid, brain, liver, cerebrospinal fluid, cerebrolysin, glia depressing factor

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Introduction

In the last thirty years particular attention was paid to tryptophan research and findings related to the role of kynurenine metabolites in neurodegenerative and neuroinflammatory processes revealed remarkable evidence and importance for its functional involvement. Kynurenic acid (KYNA), an endogenous metabolite of the kynurenine pathway of tryptophan degradation, is a well known endogenous antagonist of three glutamate ionotropic excitatory amino acid receptors, i.e. N-methyl-*D*-aspartate (NMDA), alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and kainate[1,2] and of the nicotine cholinergic subtype alpha-7 receptors.[3] Suggested KYNA's anticonvulsive and neuroprotective properties have been demonstrated by various pharmacological approaches[1,4] and there is evidence that KYNA improves cognition and memory.[5] Conversely, it has also been demonstrated that KYNA interferes with the working memory[6] and an impairment of both cholinergic and glutamatergic neurotransmission due to KYNA actions has been proposed in neuropsychiatric and neurological diseases.[3,7]

Interestingly, it has also been shown that within investigated tryptophan metabolites KYNA enhances the oxygen consumption in rat heart mitochondria, in an *in vitro* study,[8] and therefore an essential role of KYNA for the cell function of the myocardium can not be excluded.

An enhancement of KYNA levels in the brain and/or serum has been found under various experimental pathological conditions, as demonstrated in the animal models of asphyxia,[9] dystonia,[10] or epilepsy.[11] KYNA's involvement in human neurodegenerative and neuroinflammatory processes has been well documented,[1,12] thus the increased KYNA metabolism is seen in Alzheimer's patients,[13] in patients with subcortical sclerotic encephalopathy,[14] in patients infected with HIV-1 virus,[15,16] in patients with Schizophrenia[17] and also in elderly human subjects.[18] KYNA is synthesized by irreversible transamination of L-kynurenine.[20,21] In organs of mammalians several aminotransferases convert L-kynurenine into KYNA.[21,22] In peripheral tissues of rats there are at least four types of proteins which are capable of catalysing the kynurenine-2-oxoacids transamination reaction to produce KYNA.[21,22] In human and rat brain tissues kynurenine aminotransferase I, II and III (KAT I KAT II and KAT III) were described, and significant differences in respect to the regional brain distribution were revealed.[13,23–25] KAT I, KAT II and KAT III are capable of synthesizing KYNA, and show different catalytic characteristics,[23–29] which suggests that substantially KAT II and probably KAT III act under physiological conditions, whereas KAT I may have a particular importance in pathological conditions, like in microglia activation. There is also data indicating that human KAT I is a multifunctional enzyme and might play a role in KYNA synthesis even under physiological conditions.[30]

Research on KAT cellular localisation indicates that in the rat brain KAT II has a preferential astrocytic and microglia localisation.[31] Furthermore, Guillemin and co-workers demonstrated the presence of KAT(s) mRNA in human astrocytes.[32] Studies using *in situ* hybridisation have shown that KAT I mRNA activity is expressed in mitochondria of neurons and glial cells and also in the cytosol of choroid plexus epithelial cells.[33]

The aim of this study was to investigate the probability that endogenous compounds, e.g. proteins/cells, present in the tissues or body fluids might exert the ability to influence KYNA synthesis. Since rat liver exerts a very dominant capacity to synthesize KYNA we used the measurement of KAT activity in the liver as a fundament to search the effect of these endogenous compounds. For this purpose KYNA formation in the reaction mixture, which contained rat liver homogenate and homogenate of piglet tissue or rat tissue or human tissue or human CSF, was investigated.

Material and Methods
Chemicals
L-kynurenine, KYNA and pyridoxal-5'-phosphate were purchased from Sigma. [^{3}H]L-kynurenine (specific activity, 41 Ci/mmole) was purchased from Amersham, England. All other chemicals used were of the highest purity that was commercially available.

Biological materials
Piglets
Piglets (Veterinary Medical University of Vienna, Vienna, Austria) weighing 23 kilograms to 27 kilograms of body weight were used. Blood samples were collected in the morning and frozen at $-60\,°C$ until analysis. Piglets

were sacrificed, and the peripheral organs the liver, heart and lung were dissected, the brain was removed and the frontal cortex was extracted and samples were frozen at −60 °C until analysis. The number of piglets used was $n = 6$. Fresh liver tissue of adult pigs from a commercial slaughter-house was obtained and stored at −60 °C until analysis, the number of samples was $n = 3$.

Rats
Male Sprague-Dawley rats (Research Institute for Animal Breeding, Himberg, Austria) weighing 200 grams to 220 grams of body weight were used. The animals were housed in groups of four to five per cage, in a room with controlled light/dark cycle (12 h light/12 h dark), and were given free access to laboratory food and top water. Rats were sacrificed in the morning, the liver was dissected, the brain immediately removed and the frontal cortex extracted and samples were frozen at −60 °C until analysis. Blood samples were collected and frozen at −60 °C until analysis. The number of rats used was $n = 10$.

Human material
Post mortem human samples of frontal cortices of normal subjects, aged between 40 and 45 years, were received frozen from the Institute of Neurology, Medical University Vienna, Vienna, Austria and stored at −43 °C until analysis. The number of human samples used was $n = 10$. Post mortem human heart samples (ventricular tissue) of normal subjects ($n = 5$), aged between 30 and 38 years, were received frozen from the Department of Pathology, Medical University Vienna, Vienna, Austria and stored at −43 °C until analysis.

Human CSF and serum samples
CSF and serum samples of normal human subjects were obtained from the Medical Laboratory of the Neuropsychiatric Hospital Mauer, Amstetten/Mauer, Austria. Twenty five individuals of a larger series of patients with severe headache who underwent lumbar puncture to exclude subarachnoidal haemorrhage or meningitis were selected as normal subjects for this study after CSF investigation, neuroimaging and clinical follow up. The number of serum used was $n = 8$ of 25. Twenty three CSF samples were from patients diagnosed as relapsing remitting Multiple Sclerosis (MS) according to international accepted MS criteria and 5 CSF samples were from patients diagnosed as Neuroborreliosis. All samples were provided from the Neurological Department of the Neuropsychiatric Hospital Landesklinikum Mauer-Amstetten, Austria. Ages ranged between 25 years and 54 years for control subjects and 23 years and 48 years for MS patients and 45 years and 59 years for patients with Neuroborreliosis, respectively. Samples of CSF and serum were coded and the study was carried out according to Lower Austrian Ethical Regulations.

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

Neuroradiological investigations
Magnetic resonance tomography (MRT) was carried out in all patients with suspected MS and Neuroborreliosis and computed tomography (CT) and/or MRT was performed in headache patients. No patients with strokes were included in this study.

Preparation of samples
Sample collection
Samples of human CSF and human serum of control subjects and samples of CSF of MS and Neuroborreliosis subjects were collected in 1 mL aliquots and stored at −43 °C until analysed.

CSF centrifugation
A sample of 900 µl of CSF from patients with Neuroborreliosis were centrifuged at 1000 rpm for 5 min, the supernatant of 870 µl was carefully removed, labeled as CSF supernatant fraction (CSF-Sup), and the rest of 30 µl with sediment was resuspended into 100 µl of homogenization buffer (i.e. the fraction of 30 µl was 4.3 times diluted) and labeled as CSF-sediment fraction (CSF-Sed). The obtained two fractions were immediately tested for the ability to block rat liver KAT I activity.

Preparation of homogenate

The organ samples were homogenised in an ice bath in 10 volumes (wt/vol) of 5 mM Tris-acetate buffer pH 8.0 containing 50 µM pyridoxal-5'-phosphate and 10 mM mercaptoethanol (homogenisation buffer) and the homogenates obtained were immediately used for KAT I and II activities measurement.

Measurement of KYNA

The measurement of KYNA was performed according to Shibata et al[36] and Swartz et al[37] with modification as described by Baran et al[13] Briefly, the tissues (liver, heart, lung, and brain frontal cortex) were homogenised in an ice bath in 10 volumes (wt/vol) with H_2O and immediately mixed with 0.2 M HCl (vol/vol), whereas serum samples were mixed with 0.2 M HCl (vol/vol) and centrifuged for 20 min, at 14,000 rpm. The supernatant obtained was applied to a Dowex 50 W cation exchange column pre-washed 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[38] and was quantitated by a high performance liquid chromatography (HPLC) system coupled with fluorescence detection.

Determination of KAT I and KAT II activities

KAT I and KAT II activities were measured using a radio-enzymatic assay described by Schmidt et al[27] with minor modification. In brief, the reaction mixture contained homogenate and homogenisation buffer or second homogenate, or serum or CSF, 100 µM 1.175 µCi/µmol [³H]L-kynurenine (or 100 µM L-kynurenine), 1 mM pyruvate, 70 µM pyridoxal-5'-phosphate and 150 mM 2-amino-2-methyl-l-propranol buffer pH 9.6 for KAT I or 150 mM Tris-acetate buffer pH 7.0 for KAT II, in a total volume of 200 µl. After incubation for 16 hours 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 50 W cation-exchange column[38] and quantified by liquid scintillation spectrometry. Blanks were prepared by boiling samples of homogenate for 15 minutes before adding the reaction mixture. In separate experiments using 100 µM L-kynurenine the KYNA formed was quantified by HPLC system.

Linearity of assay with different times and piglet tissue amounts

The incubation time and the tissue amount with respect to linearity of KAT I and KAT II activities measurement in homogenate of rat or piglet organs were evaluated. KAT I and II activities of rat brain and liver, or piglet brain, liver and heart showed linearity between 1 mg and 20 mg for brain and heart, and between 0.125 mg and 0.75 mg for liver in the incubation mixture. KAT I and II activities measurement was linear up to 16 hours of incubation time.

Experimental design
Measurement of KYNA

The KYNA content was measured in the serum and in the brain (frontal cortex) of piglet, rat and human samples.

Measurement of KAT I and KAT II activities in piglet, rat and human

KAT I and KAT II were determined: in the homogenate of piglet, rat or human brain (frontal cortex, 75 µl of homogenate prepared in 20 volumes, wt/vol); in homogenate of rat or human heart (75 µl of homogenate prepared in 20 volumes, wt/vol); in the homogenate of rat, piglet or pig liver (25 µl of homogenate prepared in 100 volumes, wt/vol) and in the homogenate of piglet lung (25 µl of homogenate prepared in 100 volumes, wt/vol).

KAT activities in the mixture of two different homogenates

KAT I and KAT II activities were measured in the reaction mixture containing rat liver and rat brain homogenates, or rat liver and piglet brain homogenates, or rat liver and piglet liver homogenates, or rat liver and human brain homogenates.

Influence of human CSF on rat liver KAT I and II activities

Influence of human CSF of normal control subjects on the rat liver KAT I and KAT II activities was investigated. The effect of human CSF of MS patients on rat liver KAT I activity was compared to effect of human

CSF of normal control subjects. Then, the effect of boiled human CSF of control subjects and boiled CSF of MS patients on KAT I activity was researched.

Influence of CSF-sediment (CSF-Sed) and CSF-supernatant (CSF-Sup) fractions on KAT I

Rat liver KAT I activity in the presence of CSF-Sed or CSF-Sup fractions, obtained after CSF centrifugation, using two doses of 35 µl and 75 µl, respectively, was researched.

Data analyses

All data are given as means ± S.E.M. For statistical analyses, the one-way ANOVA-test and a Student's t-test were applied, respectively. Each sample was determined in duplicate or triplicate. Asterisks indicate a significant difference: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ compared to the used control, respectively.

Results
KYNA level

In piglet serum the KYNA content was at a low nanomolar range, i.e. 3.39 ± 0.09 nM, and the KYNA level was approximately eight times lower than in the human serum and twenty six times lower than in the rat serum (Table 1). The KYNA levels found in rat and human serum corresponded well with previously published data.[1,11,18,39]

In the piglet frontal cortex KYNA level was found at low nanomolar concentration (4.55 ± 0.09 nM, $n = 6$) (data not shown).

KAT I and KAT II activities

The KAT I and KAT II activities in the piglet frontal cortex were higher than in human frontal cortex, i.e. approximately three times higher for KAT I and four times higher for KAT II, but lower than in the rat frontal cortex, i.e. five times lower for KAT I and ten

Table 1. Kynurenic acid (KYNA) levels in the serum of different species.

Species	KYNA in serum [fmol/µl]
Piglet	3.39 ± 0.09 (6)
Rat	89.72 ± 1.40 (10)
Human	27.91 ± 0.99 (8)

All data is given as means ± SEM. Number of samples is given in parentheses.

times lower for KAT II (Table 2). No activities (very low or even negative values) of KAT I and KAT II were found in the piglet liver, heart and lung, whereas in the rat liver KAT I and KAT II activities were very high. In the adult pig liver, the KAT I and KAT II activities were not or only marginal detectable (data not shown).

KAT I and KAT II activities in mixtures of two different homogenates

KAT I and KAT II activities of rat liver or rat brain homogenate or a mixture of the two different homogenates are shown in Table 3. The activity of KAT I and KAT II in the reaction mixture containing rat liver and brain homogenates was comparable to the total activity of both homogenates if incubated separately. In contrast, the piglet brain, human brain or piglet liver homogenate significantly lowered the rat liver KAT I and KAT II activities. Piglet brain homogenate reduced rat liver KAT I by 54.5% of the control ($P < 0.001$) and KAT II by 76.0% of the control ($P < 0.01$), and human brain homogenate reduced the rat liver KAT I by 70% of the control ($P < 0.01$) and KAT II by 72% of the control ($P < 0.01$). Piglet liver homogenate reduced the rat liver KAT I by 50.8% of the control ($P < 0.001$) and KAT II by 69.1% of the control ($P < 0.01$). Boiled piglet liver homogenate lost the ability to block the rat liver KAT activities, thus, KAT I was 98.6% and KAT II 99.1% of the control, respectively. The reduction of rat liver KAT I and II activities was more pronounced for

Table 2. Activities of kynurenine aminotransferase I and II (KAT I and KAT II) in piglet, rat and human tissues.

Species/ Organ		KAT I [pmol/mg wet weight tissue/h]	KAT II [pmol/mg wet weight tissue/h]
Piglet	Brain	2.54 ± 0.36 (6)	2.34 ± 0.42 (6)
	Liver	nd (5)	nd (5)
	Heart	nd (5)	nd (5)
	Lung	nd (5)	nd (5)
Rat	Brain	12.72 ± 0.78 (8)	22.71 ± 1.27 (8)
	Liver	369.49 ± 30.18 (10)	$2,109.4 \pm 141.4$ (10)
Human	Brain	0.919 ± 0.021 (5)	0.538 ± 0.020 (5)
	Heart	0.732 ± 0.030 (5)	0.451 ± 0.021 (5)

All data is given as means ± SEM. As a region of the brain the frontal cortex was used. Number of animals and number of human subjects are given in parentheses; nd not detectable and/or even negative value.

Table 3. Kynurenine aminotransferase I and II (KAT I and KAT II) activities in rat liver homogenate: influence of rat brain, piglet brain or human brain.

Homogenate	KAT I [dpm/reaction mixture] [% of control]	KAT II [dpm/reaction mixture] [% of control]
Rat liver (Control, CO)	2966.6 ± 181.5 (5) **100%**	19929.2 ± 1036.2 (5) **100%**
Rat brain	2240.0 ± 89.5 (5)	3654.6 ± 123.9 (5)
Rat liver + rat brain	4931.6 ± 191.4 (5)	23212.06 ± 544.1 (5)
Rat liver + piglet brain	1617.8 ± 55.4*** (5) **54.5% of CO**	15150.8 ± 856.9** (5) **76.0% of CO**
Rat liver + piglet liver	1506.36 ± 47.6*** (5) **50.8% of CO**	13762.0 ± 814.0** (5) **69.1% of CO**
Rat liver + piglet liver boiled	2925.4 ± 214.5 (5) **98.6% of CO**	19755.5 ± 930.9 (5) **99.1% of CO**
Rat liver + human brain	2077.27 ± 52.7** (5) **70% of CO**	14346.0 ± 669.5** (5) **72% of CO**

All data is given as means ± SEM. KAT I and KAT II activities were assayed as described in Material and Methods; Number of independent measurements are given in parentheses. KAT I and KAT II activities expressed in [pmol/mg wet tissue weight/h] are: for rat liver KAT I is 337.3 ± 25.5 (5) and KAT II is 2292.1 ± 119.2 (5), and for rat brain KAT I is 17.17 ± 0.69 (5) and KAT II is 28.0 ± 0.95 (5), respectively. Significance of differences: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ vs. corresponding control.
Abbreviations: nd, not detectable; CO, control.

KAT I than for KAT II and in the presence of piglet brain homogenate than of human brain homogenate, and being dependent on the amount of homogenate added (data not shown).

Effect of human CSF on rat liver KAT activities

CSF of human control subjects reduced rat liver KAT I to 20.5% of control ($P < 0.001$) and KAT II was 103% of control (Table 4). The reduction of rat liver KAT I by human CSF was dependent on the amount of CSF added. Measurement of KAT I and KAT II in CSF samples revealed only marginal activity, which is comparable to our previously published data.[18,41]

The effect of CSF of MS patients on rat liver KAT I activity is shown in Figure 1. The reduction of rat liver KAT I activity was more pronounced by CSF of the control subjects than by the CSF of the MS patients. Boiled CSF of MS patients and control subjects in part lost the ability to block the rat liver KAT I activity.

Table 4. Alterations of rat liver kynurenine aminotransferase I and II (KAT I and KAT II) activities in the presence of cerebrospinal fluid (CSF) of normal human subjects.

Homogenate	KAT I [pmol/mg wet tissue weight/h] [% of control]	KAT II [pmol/mg wet tissue weight/h] [% of control]
Rat liver (Control, CO)	311.6 ± 39.6 (4) **100%**	1914.8 ± 134.8 (4) **100%**
Rat liver + CSF 75 μl	63.9 ± 21.6 (4) **20.5% of CO**	1946.9 ± 154.1 (4) **103% of CO**
Rat liver + CSF 30 μl	112.4 ± 7.0 (3) **36.1% of CO**	nd
Rat liver + CSF 10 μl	233.4 ± 12.8 (3) **74.9% of CO**	nd

All data is given as means ± SEM. KAT I and KAT II activities were assayed as described in Material and Methods; Number of independent measurements are given in parentheses. KAT I and KAT II activities of CSF were: 121.3 ± 27.5 (4) and 15.1 ± 4.3 (4) [fmol/μl/h], respectively. Significance of differences: *$P < 0.05$; **$P < 0.01$; vs. corresponding control.
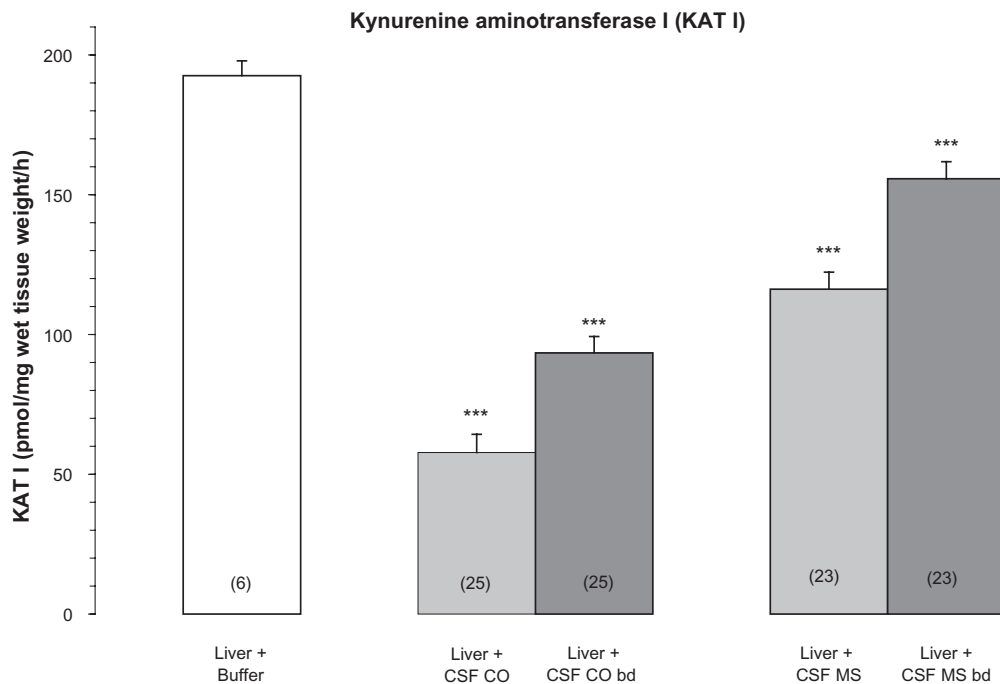**Abbreviations:** nd, not determined.

Figure 1. Effect of cerebrospinal fluid (CSF) of Multiple Sclerosis (MS) and of control subjects (CO) on rat liver kynurenine aminotransferase I (KAT I) activity. KAT I activity was assayed as described in Material and Methods. Data is expressed in [pmol/mg wet tissue weight/h] and represent means ± SEM; CSF CO bd and CSF MS bd—CSF samples after 15 min of boiling. Number of independent measurements are given in parentheses. Significance of differences: ***$P < 0.001$ vs. the corresponding control rat liver KAT I activity.

Influence of human CSF-supernatant (CSF-Sup) and CSF-sediment (CSF-Sed) fractions on rat liver KAT I activity

The effect of two different CSF fractions obtained due to centrifugation of CSF of patients with Neuroborreliosis is shown in Figure 2. Both CSF-Sup (75 µl) and CSF-Sed fractions (75 µl) significantly lowered rat liver KAT I by 46.81% and 21.64% of control ($P < 0.001$), respectively. The inhibitory effect of CSF-Sed fraction was markedly stronger compared to CSF-Sup fraction since CSF-Sed fraction was 4.3 times diluted, due to the fraction preparation procedure. The application of a lower dose of 35 µl of both fractions revealed a weaker effect and it was 67.5% and 31.5% of the control, respectively.

Discussion

From previous studies it is known that in rats and humans KAT activity is predominantly present in the liver—other organs exerting only moderate activity.[24,40] For the first time, our data demonstrated no KAT activity in porcine liver and also in other piglet peripheral organs. We also found very low KYNA levels in piglet serum (3.4 nM). Kynurenine

metabolism in piglet frontal cortex was easily detectable and KAT activities and KYNA content measured in rat and human control subjects correlated well with earlier published data.[1,11,18,39] Lack of KAT activity in piglet liver could indicate an absence of the biochemical machinery to synthesise KYNA. An alternative explanation is the presence of unknown yet endogenous components which interfere with KYNA formation.

The significant observation in our present study was that KYNA formation in rat liver, measured under standard assay condition for KAT activity, was indeed altered in the presence of porcine liver homogenate. In fact, not only the extract of piglet liver but also piglet brain lowered significantly rat liver KAT I and KAT II activities. Interestingly, no inhibition of rat liver KAT was observed in the presence of rat brain homogenate indicating a lack or very low level of inhibitory component(s). This finding does not rule out the possibility of interference because it may critically depend on the species and age. The inhibition of rat liver KAT was also seen in the presence of human brain and CSF, and serum (Baran personal information), as well. The inhibitory effect was dose dependent, it was more pronounced for KAT I than for KAT II,
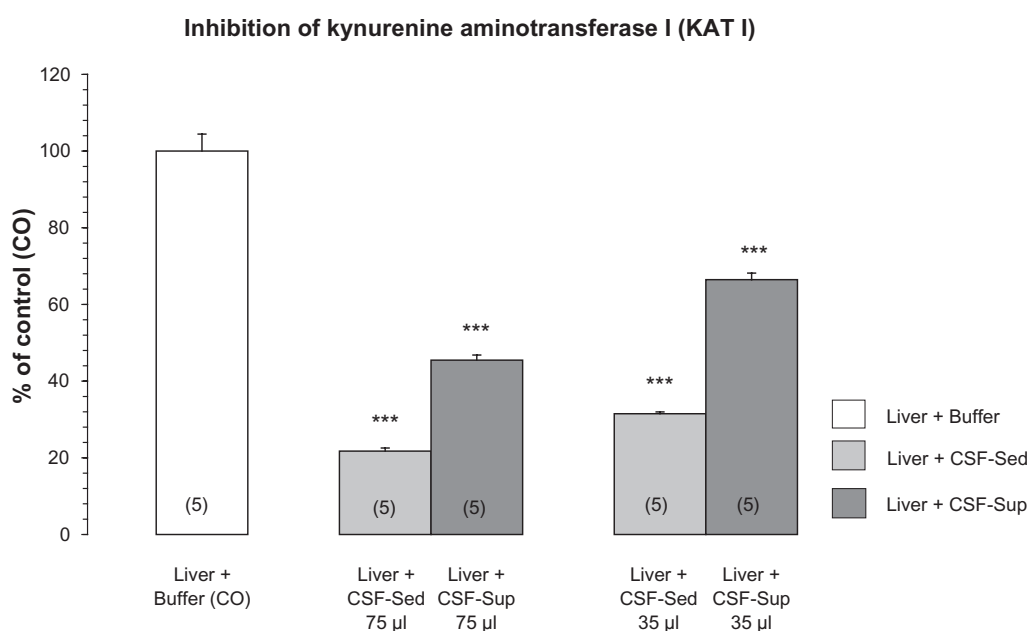
Inhibition of kynurenine aminotransferase I (KAT I)

Figure 2. Effect of different fractions of cerebrospinal fluid (CSF) of patients with Neuroborreliosis on rat liver kynurenine aminotransferase I (KAT I) activity. KAT I activity was assayed as described in Material and Methods. Fractions of CSF-sediment (CSF-Sed) and CSF-supernatant (CSF-Sup) obtained after CSF centrifugation were prepared as described in Material and Methods. Data is expressed in % of control and represent means ± SEM. Number of independent measurements are given in parentheses. Significance of differences: ***$P < 0.001$ vs. the corresponding control rat liver KAT I activity (CO) and between both application of 35 and 75 µl.

and was heat sensitive indicating an involvement of thermo-sensitive protein-like compound(s).

The coherence between a high inhibitory capacity and low KAT activity (and likely low KYNA formation) of investigated biological materials is significant. It is possible that low KAT activity measured in tissue is due to the presence of this endogenous KAT inhibiting compound(s).

The most interesting observation in our study was that the inhibitory effect of CSF of MS patients was significantly weaker compared to CSF of the human control subjects, suggesting a clinically important effect. This effect of suppressed inhibition may cause a higher KYNA synthesis and activation of glia in the CNS of MS patients. This hypothesis is supported by the fact that indeed enhanced KYNA levels in the CSF[42] and the presence of gliosis and plaque formation in the brain in the acute stage of MS patients has been reported.[49]

The inhibitory capacity of human CSF to block KAT I could play a particularly important role since CSF is produced by choroid plexus epithelial cells and KAT I mRNA activity is expressed in the cytosol of the choroid plexus epithelial cells.[33] It is questionable if choroid plexus epithelial cells or other cells like lymphocytes or even neurons are involved in the formation of those inhibitors. In that respect, we found

that the inhibitory effect was notably present by using CSF-Sed fraction and this data strongly indicated the involvement of proteins/cells of CSF.

Our recently published data has demonstrated that Cerebrolysin, which contains an extract of porcine peptides, has the ability to block not only rat liver KAT activities but also KAT activities of the rat and human brain.[25] In this study we proposed that Cerebrolysin induced KAT inhibition might affect glia proliferation, too.[25] In line with our suggestion a study on transgenic mouse model of Alzheimer's disease showed that Cerebrolysin treatment significantly ameliorated cerebrovascular amyloidosis, perivascular and interstitial microgliosis, and furthermore astrogliosis was markedly reduced after Cerebrolysin treatment, as well.[43] Álvarez et al[44] demonstrated that Cerebrolysin reduced amoeboid microglia activity indicating that this porcine peptide extract has the ability to attenuate microglia activation. Although the therapeutic efficacy of Cerebrolysin has been proposed due to a neurotrophic activity,[45] we suggested that KAT inhibition might contribute to an attenuation of microglia proliferation.[25]

In this study, for the first time, our accumulated findings demonstrate the differences in the capability of endogenous components of various species and various physiological and pathological conditions to block KAT

activity and we propose the presence of glia depressing factor (GDF). The presence of this factor is abundant, since an inhibitory effect on KAT was observed in several homogenates of different organs, by CSF, and also in human serum. In the investigated species, significantly different inhibitory capacities of brain homogenate have been found suggesting species dependent different distribution i.e. very high in pigs, lower in humans and very moderate in rat brain homogenate.

A remarkable enhancement of rat brain KYNA metabolism i.e. enhancement of KAT activity and an increased glia proliferation during the aging process[24,31] could be due to the lowering of GDF levels with aging and this needs to be proved through further investigation. On the other hand high GDF levels and low KAT activity (due to KAT blockade) may lead to a "re-direction" of the kynurenine pathway and synthesis of quinolinic acid, which acting as an endogenous agonist of NMDA receptors, is significantly involved in the synaptogenesis during brain development.[46] However, under certain conditions, the "re-direction" of the kynurenine pathway might be associated with overproduction of quinolinic acid and induction of neurodegenerative processes and/or epileptic activities.[1,19]

Morgan et al showed that food restriction decreased the transcription of GFAP in ageing rats and lowered microglia activation during ageing,[47] therefore selected food consumption might have a significant impact on many physiological and pathological processes in humans.

In summary, a remarkably low KYNA metabolism was found in piglet periphery and CNS, comparing to rat and human organs. For the first time we demonstrated that porcine tissues extract and human CSF, serum or brain extract shows the ability to block significantly KAT I and partly KAT II activities. We propose the presence of a glia depressing factor (GDF), which might have a significant impact not only on regulation of KYNA metabolism but also on regulation of glia/astroglia activity respectively and glia proliferation. Apart from the first contribution to understand the mechanism(s) involved in the regulation of KYNA metabolism, our observations might have potential diagnostic implications.[48] The composition and structure of GDF and the mechanism(s) of action in mammalians, especially the role of GDF during development, aging and pathological conditions needs to be elaborated.

Disclosure
This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors report no conflicts of interest.

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