Peripheral Kynurenine Metabolism in Focal Dystonia

Zsuzsanna Hartai[a], Peter Klivenyi[a], Tamas Janaky[b], Botond Penke[b], Laszlo Dux[c] and Laszlo Vecsei[a,d,*]

[a] *Department of Neurology, University of Szeged, 6 Semmelweis u., H-6725 Szeged, Hungary;* [b] *Department of Medical Chemistry, University of Szeged, 8 Dom ter, H-6720 Szeged, Hungary;* [c] *Department of Medical Biochemistry, University of Szeged, 9 Dom ter, H-6720 Szeged, Hungary;* [d] *Neurology Research Group of Hungarian Academy of Sciences and University of Szeged, 6 Semmelweis u., H-6725 Szeged, Hungary*

Abstract: Substantial evidence indicates that neuroactive kynurenine metabolites play a role in the normal physiology of the human brain, and are involved in the pathology of neurodegenerative disorders such as Parkinson's disease and Huntington's disease. A side-arm product of the pathway, kynurenic acid (KYNA), which is synthesized by the irreversible transamination of kynurenine (KYN) by kynurenine aminotransferases (KAT I and KAT II), is an excitatory amino acid receptor antagonist. In the present study, we measured the level of KYNA and the activities of the biosynthetic enzyme isoforms KAT I and KAT II in the plasma and in the erythrocytes (RBCs) of patients with cervical dystonia or blepharospasm and in age-matched controls. The KAT I and KAT II activities were significantly lower in the plasma of the patients in both subgroups. In the RBCs, only the KAT I activity was elevated significantly. The KYNA concentration was unchanged in both type of patients. These data support the contribution of an altered kynurenine metabolism to the pathogenesis of focal dystonia.

Key Words: Cervical dystonia, blepharospasm, kynurenine aminotransferase, kynurenic acid, excitotoxicity.

1. INTRODUCTION

The dystonias are a group of conditions characterized by the presence of sustained involuntary muscle contractions that can lead to twisting or repetitive movements and abnormal postures. Focal dystonia affects only a single body part, with symptoms varying from permanent (e.g. cervical dystonia) to task-specific (e.g. musician's cramp). The exact causes of focal dystonia have yet to be determined. Possible causative factors have been identified along the sensorimotor pathway, including postural constraints of the hand, abnormal co-contractions of the muscles due to reciprocal inhibition in the spinal cord, cortical remapping, deficiencies in sensorimotor integration and perceptual deficits [1]. Less is known about neurochemical changes. Although dystonias are thought to be a disease of the central nervous system (CNS), neurochemical alterations may be present in peripheral tissues, as we earlier demonstrated in Parkinson's disease, Leber's disease and multiple sclerosis [2,3,4].

In mammalian cells, the essential amino acid tryptophan is degraded to nicotinic acid primarily *via* the kynurenine (KYN) pathway (Fig. (**1**)). An intermediate product of the KYN metabolism is quinolinic acid (QUIN), which is an N-methyl-D-aspartate (NMDA) receptor agonist, while the side-arm product kynurenic acid (KYNA) is a broad-spectrum antagonist at the three ionotropic excitatory amino acid receptors, and a non-competitive antagonist at α7- nicotinic acetylcholine receptors [5,6]. It has been demonstrated that KYNA may act as a potent neuroprotective and anticonvulsive compound in various conditions [7]. The two enzyme isoforms that are primarily responsible for the synthesis of KYNA are kynurenine aminotransferase I and II (KAT I and KAT II). These enzymes have different pH optima (pH 9.6 for KAT I, and pH 7.4 for KAT II) [8,9], and exhibit different catalytic characteristics. Metabolites of this pathway have been shown to be involved in many diverse physiological and pathological processes, including movement disorders.

Only limited data are available regarding the KYN metabolism in dystonia. In the plasma of patients with primary generalised dystonia, the concentration of KYN was reported to be unchanged [1]. In a genetic model of dystonia, an elevated brain KYNA level was demonstrated [11].

As far as we are aware, the KYN metabolism in the blood in focal dystonias has not been reported previously. No data are available on the KYNA level and on the activities of KAT I and KAT II in the blood in these disorders.

The aim of the present study was to determine the activities of KAT I and KAT II, and to measure the concentration of the neuroprotective KYNA in the plasma and RBC in order to establish whether this system is involved in the pathogenesis of focal dystonia.

2. MATERIALS AND METHODS

2.1. Patients

The study was approved by the Human Investigation Review Board of the University of Szeged and informed consent was obtained from each of the patients who participated in the study.

KAT activities were measured on 7 patients with cervical dystonia (2 males and 5 females, mean age 43.5 ± 4.7 years,

*Address correspondence to this author at the Department of Neurology, University of Szeged, 6 Semmelweis u., H-6725 Szeged, Hungary;
E-mail: vecsei@nepsy.szote.u-szeged.hu

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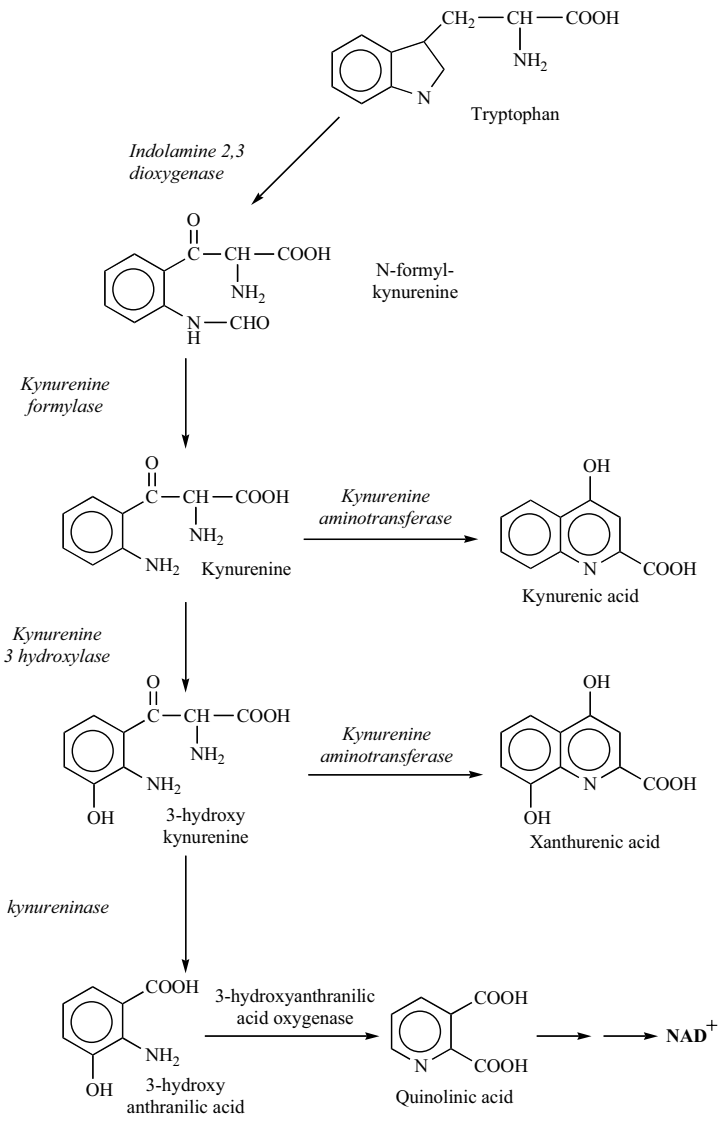

Fig. (1). The kynurenine pathway.

mean ± SD), and 11 patients with blepharospasm (2 males and 9 females, mean age 49.8 ± 5.4 years, mean ± SD). Eighteen healthy age and sex-matched volunteers (4 males and 14 females, mean age 45.1 ± 3.4 years, mean ± SD) served as controls. The diagnosis of focal dystonia was proved by the cardinal clinical signs. The patients had been on botulinum toxin medication for at least 2 years, administered at 3 monthly intervals. The patients had received the most recent botulinum toxin treatment 3 months prior to the study. None of the subjects took any other drug regularly. The blood samples were taken between 9 and 11 am, separated immediately and kept at –80 ^{o}C until measurements. None of the subjects was on a special diet.

2.2. Materials

Kynurenine sulphate, kynurenic acid, pyridoxal-5'-phosphate, 2-oxoglutarate and zinc acetate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile was from Scharlau (Madrid, Spain). Other chemicals were from different commercial suppliers and were the purest available or analytical grade.

2.3. Kynurenine Aminotransferase Activity

The activities of the KATs were assayed by the modified method of Mason [12]. After withdrawal the RBCs and plasma were frozen separately and stored at -80 °C until they were measured. The RBCs were haemolysed with distilled water. The reaction mixture contained 100 µl of the haemolysed RBC or plasma sample, 10 mM 2-oxoglutarate, 40 µM pyridoxal-5'-phosphate, 1 mM KYN in 1/15 M phosphate buffer, pH 9.6, for KAT I measurements and pH 7.4 for KAT II measurements. No KYN was added to the blank. After incubation for 1 h at 37 °C, the reaction was terminated by the addition of boric acid. The enzyme activity was de-

tected spectrophotometrically at 333 nm. Haemoglobin and total plasma protein were used to normalise the results, which were expressed as pmol/mg Hb/h (for RBC samples) and pmol/mg protein/h (for plasma samples). The method was validated using active compound.

2.4. Determination of Kynurenic Acid

The endogenous KYNA concentrations in the RBCs and plasma of the patients and controls were measured on the basis of the method of Baran *et al*. [13]. The RBC were haemolysed with distilled water. Perchloric acid was added to the plasma or haemolysed RBCs to precipitate proteins. The sample was centrifuged at 6000 rpm for 10 min. The supernatant was added to 600 mg of Dowex 50W cation-exchange resin treated 3 times with 5 ml of 0.1 M HCl. The sample was vortexed for 3 min, and HCl was then added. After vortexing, the supernatant was discarded and the KYNA was eluted in 40 ml of water. The eluate was immediately frozen to -80 °C and lyophilised under vacuum. Prior to analysis, the sample was dissolved in 240 µl of distilled water. KYNA was quantitated by high-performance liquid chromatography (Agilent 1100 HPLC, Waldbroon, Germany), with fluorescence detection according to the method of Swartz *et al*. [14]. Briefly, the sample was applied onto a Hypersil 5 ODS HPLC column (150 x 4 mm, Thermo-Separation, Bellefonte, PA, USA), and chromatographed isocratically at a flow rate of 1 ml/min with a mobile phase consisting of 0.2 M zinc acetate and 5% acetonitrile, pH 6.2. KYNA was detected by a fluorescence detector (excitation: 344 nm, and emission: 398 nm). The method was validated using active compound.

2.5. Statistical Analysis

All assay data were expressed as means ± standard error of the mean (x ± SEM). One-way ANOVA followed by Fisher's LPSD test was used to determine significant differences between groups. A $p < 0.05$ was considered statistically significant.

3. RESULTS

The activities of KAT I and KAT II and the concentration of KYNA are shown in Fig. (**2**) and Fig. (**3**). The activities of both KAT I and KAT II were decreased in the plasma of patients with cervical dystonia and blepharospasm. There was no significant change in the concentration of KYNA in these patients.

A significantly elevated activity of KAT I was detected in the RBCs of patients with cervical dystonia or blepharospasm, while the KAT II activity and the KYNA concentration remained unchanged.

4. DISCUSSION

The present study demonstrates that the KAT activities were altered in the plasma and in the RBCs in patients with focal dystonia. No evidence is available that botulinum toxin treatment has any effect on the KYN metabolism. In the human brain, two discrete KAT isoforms (KAT I and KAT II)

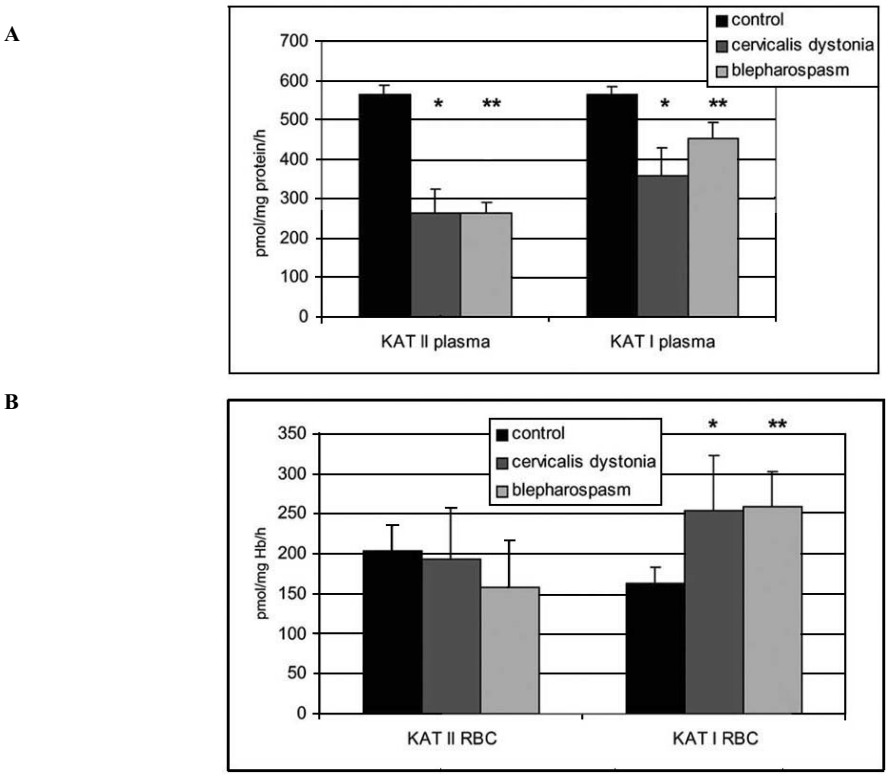

Fig. (2). KAT I and KAT II activities in the (A) plasma (pmol/mg protein/h) and in (B) red blood cells (pmol/mg Hb/h) of patients with focal dystonia. Data are means ± SEM. *p<0.01 compared to the control group.

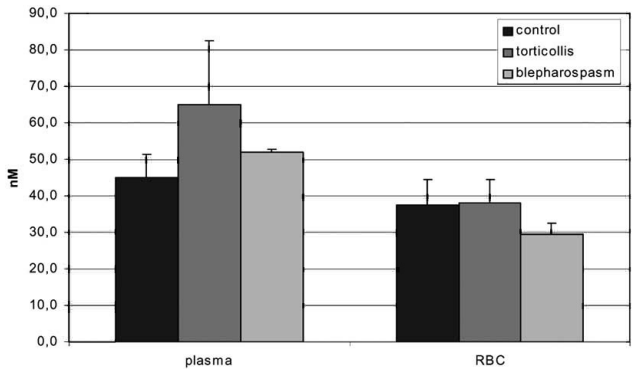

Fig. (3). Kynurenic acid concentrations (nM) of plasma and red blood cells in patients with focal dystonia. Data are means ± SEM. *$p<0.01$ compared to the control group.

catalyse the irreversible transamination of KYNA from KYN [8]. The enzymes are mainly localised in the astrocytes, in very close proximity to glutamatergic synapses, and the newly synthesised KYNA readily enters the extracellular compartment, and modulates the glutamatergic tone [5]. We have demonstrated decreased KAT I and KAT II activities in the plasma. This metabolic hypoactivity does not result in a decreased level of KYNA, which is probably compensated from other sources, such as the liver, the kidney or even the brain. No data are available regarding the KYN metabolism in these organs in focal dystonia. On the other hand, we detected elevated KAT I activity in the RBCs, which probably does not contribute significantly to the KYNA synthesis since under physiological circumstances, KAT II is responsible for 75% of the KYNA synthesis in the human brain [9]. The reason why the peripheral KYN metabolism is altered in a disease of the central nervous system is not clear. We were previously able to demonstrate similar peripheral changes in multiple sclerosis and in Parkinson's disease. There appear to be no data on the central KYN metabolism in focal dystonia. The interplay of the KYN metabolites between the brain and blood compartments has recently come increasingly into the centre of interest, but only a few data are to be found on the simultaneous changes in neurological diseases. In ALS, the KYNA concentration in the cerebrospinal fluid (CSF) is increased, while the serum KYNA level is decreased [15]. In Alzheimer's disease elevated CSF and serum KYNA levels have been reported [16]. In complex partial seizures, a decreased concentration of KYNA was measured in the serum, while in the CSF it was unchanged [17]. These data indicate a probable correlation between the blood and brain KYNA changes. Under physiological conditions, the transport of KYNA from the blood to the brain is restricted, and thus all the peripheral metabolic changes are probably ineffective. The CNS discharges KYNA *via* a probenecid-sensitive organic acid transport system [18]. These findings suggest that KYNA synthesis in the CNS does not depend on the plasma KYNA level, but it can affect the plasma KYNA changes.

These alterations may be explained by the release of excitotoxic molecules from the CNS or by systemic immunological stimulation. These compounds have not yet been identified, but could be quinolinic acid and/or 3-hydroxykynurenine. In view of the small number of patients included in this study, these conclusions must be regarded as limited.

Our results have provided additional evidence that a CNS disorder can cause peripheral neurochemical alterations, which might be helpful for a deeper understanding of the disease mechanism. Furthermore, KYNA analogues, which could be added systemically, and readily penetrate the blood-brain barrier, may present novel therapeutic approaches.

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REFERENCES

[1] Lim, V.K.; Altenmuller, E.; Bradshaw, J.L. *Hum. Mov. Sci*., **2001**, *20*, 875.
[2] Hartai, Z.; Klivenyi, P.; Janaky, T.; Penke, B.; Dux, L.; Vecsei, L. *J. Neurol. Sci*., **2005**, *239*, 31.
[3] Klivenyi, P.; Karg, E.; Rozsa, Cs.; Horvath, R.; Komoly, S.; Nemeth, I.; Turi, S.; Vécsei, L**.** J. *Neurol. Neurosurg. Psychiatry*, **2001**, *70*, 359.
[4] Hartai, Z.; Klivenyi, P.; Janaky, T.; Penke, B.; Dux, L.; Vecsei, L. *Acta Neurol. Scand*., **2005**, *112*, 93.
[5] Nemeth, H.; Toldi, J.; Vecsei, L. *Curr. Neurovasc. Res*., **2005**, *2*, 249.
[6] Hilmas, C.; Pereira, E.F.R.; Alkondon, M.; Rassoulpour, A.; Schwarcz, R.; Albuquerque, E.X. *J. Neurosci*., **2001**, *21*, 7463.
[7] Stone, T.W.; Darlington, L.G. *Nat. Rev. Drug Discov*., **2002**, *1*, 609.
[8] Guidetti, P.; Okuno, E.; Schwarcz, R. *J. Neurosci. Res*., **1997**, *50*, 457.
[9] Okuno, E.; Schmidt, W.; Parks, D.A.; Nakamura, M.; Schwarcz, R. *J. Neurochem*., **1991**, *57*, 533.
[10] Muller, U.J.; Frick, B.; Winkler, C.; Fuchs, D.; Wenning, G.K.; Poewe, W.; Mueller, J. *Mov. Disord*., **2005**, *20*, 1663.
[11] Richter, A.; Loscher, W.; Baran, H.; Gramer, M. Brain Res. *Dev. Brain Res*., **1996**, *92*, 111.
[12] Mason, M. *J. Biol. Chem*., **1954**, *211*, 839.
[13] Baran, H.; Gramer, M.; Hönack, D.; Löscher. W. Eur. *J. Pharmacol*., **1995**, *286*, 167.
[14] Swartz, K.J.; Matson, W.R.; MacGarvey, U.M.; Ryan, E.A., Beal, M.F. *Anal. Biochem*., **1990**, *185*, 363.
[15] Ilzecka, J.; Kocki, T.; Stelmasiak, Z.; Turski, W.A. *Acta Neurol. Scand*., **2003**, *107*, 412.
[16] Baran, H.; Jellinger, K.; Deecke, L. *J. Neural. Transm*., **1999**, *106*, 165.
[17] Heyes, M.P.; Saito, K.; Devinsky, O.; Nadi, N.S. *Epilepsia*, **1994**, *35*, 251.
[18] Moroni, F.; Russi, P.; Lombardi, G.; Beni, M.; Carla, V. *J. Neurochem*., **1988**, *51*, 177.

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