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AGMATINASE AND HUMAN CATIONIC AMINO ACID TRANSPORTER 1 IN MOOD DISORDER: WHAT´S UNDER THE MICROSCOPE?

DUYGUDURUM BOZUKLUKLARINDA AGMATİN VE İNSAN KATYONİK AMİNO ASİT TRANSPORTER 1: MİKROSKOBUN ALTINDA NE VAR?

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Abstract

Agmatine may act as a neurotransmitter or neuromodulator. Behaviorally, agmatine exerts antidepressant-like effects. The enzyme agmatinase degrades and thereby inactivates agmatine. The gene coding for human agmatinase is located on chromosome 1p36, a gene locus which has been linked to bipolar disorder and major depression. However, the enzyme has not yet been studied in detail in the context of neuropsychiatric diseases. We analyzed agmatinase protein expression in postmortem hippocampi of individuals with affective disorders. Agmatinase protein was detected in a subset of interneurons in the hippocampus and other brain regions. In depressive patients the number and the numerical density of agmatinase-immunopositive cell bodies was strongly elevated in all regions under study (i.e. hippocampus, habenula, insular cortex and temporal cortex). Agmatine is naturally produced by the breakdown of arginine. The cellular uptake of L-arginine and other cationic amino acids (such as L-lysine and L-ornithine) is mainly mediated by cationic amino acid transporter (CAT) proteins. In patients with mood disorder there was a circumscribed decrease in the numerical density of hCAT1 immunoreactive neurons in the CA2 region of the hippocampus.

Keywords: Brain, mood disorder, immunocytochemistry, agmatinase, human cationic aminoacid transporter 1 (hCat1).

Özet

Agmantin, nöromodülatör ve nörotransmitter olarak çalışır. Davranışsal olarak agmatin, antidepresanvari etkiler uygular. Enzim olan agmatinaz, agmatini indirger ve böylece devre dışı bırakır. İnsan agmatinini kodlayan, bipolar bozukluk ve majör depresyonla bağlantılı olan bu genin konumu 1p36. kromozomdadır. Fakat bu enzim nöropsikiyatrik hastalıklar bağlamında henüz detaylı olarak incelenmemiştir. Duygusal bozuklukları olan bireylerin postmortem hipokampüsündeki agmatin protein dışavurumunu inceledik. Agmatin proteini, hipokampüs ve diğer beyin bölgelerindeki internöronlar altkümesinde saptanmıştır. Depresif hastalarda agmatin-immunopozitif hücre gövdelerinin sayısı ve sayısal çoğunluğu incelemedeki bütün kısımlarda (hipokampüs, habenula, insular korteks ve temporal korteks) fazlasıyla artmıştır. Agmatin doğal olarak arjininin kırılmasıyla/ bozulmasıyla ortaya çıkmaktadır. L-arjinin ve diğer katyonik amino asitlerin(L-lisin ve L-ornitin gibi) hücresel alınımına temel olarak katonyik amino asit transporter(CAT) proteinleri aracılık eder. Duygudurum bozukluğu olan hastalarda, hipokampüsün CA2 kısmındaki hCAT1 immunoreaktif nöronların sayısal yoğunluğunda sınırlı bir azalma vardı.

Anahtar Kelimeler: *Beyin, duygudurum bozukluğu, immünsitokimya, agmatinaz, insanda katyonik amino asit taşıyıcısı 1 (hCat1).*

1. Introduction

The diamine agmatine may serve as a precursor in polyamine synthesis. In addition, agmatine may also act as a neurotransmitter and/or neuromodulator, binding to imidazoline receptors (reviewed in Bhutada et al., 2012). Behaviourally, it exerts anti-convulsant, (Aricioglu & Altunbas 2003; Aricioglu et al., 2003; Xu et al., 2014), anti-neurotoxic (Halaris & Piletz, 2007), vasodilatory (Satriano, 2003), neuroprotective, anti-apoptotic (Kuo et al., 2011; Moretti et al., 2014), anxiolytic (Gong et al., 2006), and especially anti-depressant-like effects (Zombowski et al., 2002; Aricioglu & Altunbas 2003; Li et al., 2003; Uzbay, 2012; Freitas et al., 2014). Interestingly, several lines of evidence suggest a prominent involvement

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of agmatine in mental disorders such as schizophrenia and depression (Zombowski et al., 2002, 2003; Moinard et al., 2005; Fiori &Turecki, 2005; Krass et al., 2008; Pålsson et al., 2008; Uzbay et al., 2013) as well as suicidal behavior (reviewed in Gross & Tureck, 2013). The enzyme agmatinase (EC. 3.5.3.11) degrades and thereby inactivates agmatine. The gene coding for human agmatinase is located on chromosome 1p36, a gene locus which has been linked to bipolar disorder and major depression (Zombowski et al., 2002; McGuffin et al., 2005; Taştemir et al., 2006; Demirhan et al., 2009; Kaneva et al., 2009; Fullerton et al., 2010). Recently, we found a significantly increased agmatinase protein expression in post-mortem hippocampi of individuals with unipolar and bipolar depression (Bernstein et al., 2012). In the present report we morphometrically analyzed agmatinase protein expression in the hippocampus and three other brain regions (habenula, insular cortex and temporal cortex) of subjects with depression to learn more about the putative role of agmatinase in the pathophysiology of mood disorders. L-Arginine is a major substrate for the synthesis of agmatine (for overview, see Halaris and Piletz, 2007). In the central nervous system (CNS), L-arginine is extracted from the blood and exchanged by cells through carriers called cationic amino acid transporters (CATs). Hence, the regional distribution and cellular localization of CATs may have a significant impact on the agmatine system. CATs have recently been shown to be widely distributed throughout human brain (Jäger et al., 2013) and have been linked with unipolar depression (Holmans et al., 2007). We therefore also determined the numerical density of human (h)CAT1 immunoreactive hippocampal neurons in mood disorders.

2. Material and Methods

All brains were obtained from New Magdeburg Brain Collection. Sampling of the human brain material and asservation was done in accordance with the Declaration of Helsinki (1984), German law and approval by the local Ethics commission. Brains were collected from 12 individuals without any psychiatric or neurological disorder (four women, eight men), eleven patients with mood disorder (four women, seven men). The age range was 35–65 years (mean age 48.1 years). Of these, seven died by suicide. Five patients displayed unipolar (major) depression (UD) and six a bipolar disorder (see tables 1 and 2). All depressed patients received long-term treatment with antidepressants. In addition, four of the bipolar patients had lithium. Tissue preparation was performed as previously described in detail (Bernstein et al., 1998). 20µm thick coronal whole brain sections were used. A well-characterized, monospecific polyclonal antibody against agmatinase was employed (Krauss et al., 2006).. We used the avidin–biotin method (Vectastain-peroxidase kit) with 3,3′-diaminobenzidine as chromogen. The colour reaction was enhanced by adding 2 ml of a 0.5% nickel ammonium sulfate solution to the diaminobenzidine (Bernstein et al., 1999). To immunolocalize hCAT1 we used a monospecific, polyclonal antiserum to the hCAT protein1 (Jäger et al., 2013). Cell countings (agmatinase: hippocampus, habenula, insular cortex, temporal cortex; hCAT1: hippocampus) and fiber densities (agmatinase: habenula) were performed using the optical disector method and a counting grid as described earlier (Bernstein et al., 1998; Lendeckel et al., 2009). Data were statistically analyzed using the non-parametric U-test (Mann and Whitney).

Table 1: Demographical data for the controls (psychiatrically unaffected individuals).

Individuals without mood disorder (controls)	Age (years)	Gender	Cause of death	Duration of illness (years)	Postmortem delay (h)
1	50	m	Cardiac insufficiency	0	72
2	47	m	Cardiac and circulatory failure	0	24
3	47	m	Acute respiratory insufficiency	0	24
4	72	f	Pneumonia, pancreas carcinoma	0	24
5	51	m	Cardiac and circulatory failure, pulmonary insufficiency	0	24
6	64	m	Rupture of the aorta	0	35
7	48	m	Heart failure, arteriosclerosis	0	72
8	63	m	Sudden cardiac death	0	48
9	54	m	Pulmonary embolism	0	24
10	39	f	Cardiac insufficiency	0	48
11	40	f	Pneumonia	0	48
12	48	f	Pneumonia	0	48

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Table 2: Demographical data for the subjects with mood disorder.

Individuals without mood disorder	Age (years)	Gender	Cause of death	Duration of illness (years)	Postmortem delay (h)
Unipolar					
1	39	f	Suicide (tablets)	7	48
2	46	f	Suicide (hanging)	11	48
3	35	m	Suicide (hanging)	2	15
4	36	m	Suicide (tablets)	1	42
5	60	m	Suicide	unknown	24
Bipolar					
6	62	f	Heart failure	11	72
7	59	m	Suicide (Shooting)	24	72
8	39	m	Heart failure	14	56
9	65	f	Pulmonary embolism	25	52
10	42	m	Suicide	16	17
11	47	m	Myocardial infarction	9	24

3. Results

3.1. Agmatinase

We herein could replicate our previous observation that agmatinase is predominantly expressed in multiple interneurons (Fig. 1A) and nerve fibers (Bernstein et al., 2011). Quantitatively, we found a significant ($p<0.05$) upregulation of agmatinase expression in neuronal cell bodies and fibers of all hippocampal subfields (not shown here, as already reported in our previous communication Bernstein et al., 2012), the in subdivisons of the habenula (Fig. 2) as well as in the insular and the temporal cortex (Fig. 3).

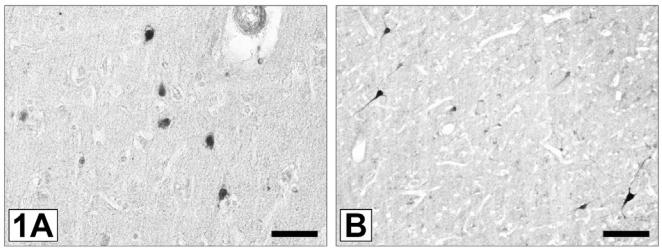

Figure 1: Immunohistochemical localization of agmatinase and hCAT1 in human brain neurons; **Figure 1A:** Agmatinase-expressing interneurons in the human temporal cortex. Bar = 35μm; **Figure 1B:** HCAT1 immunpositive neurons in the hippocampus. Bar = 70μm.

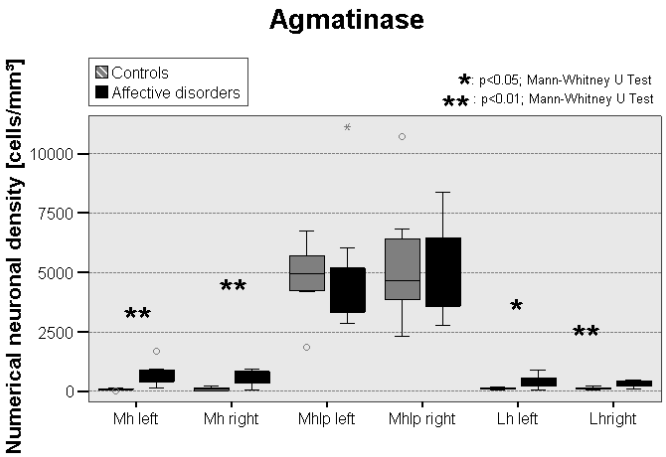

Figure 2: Numerical density of agmatinase-expressing neurons in the habenula of controls and depressed patients. Mh, medial habenula; Mhlp, medial habenula, lateral part; Lh, lateral habenula

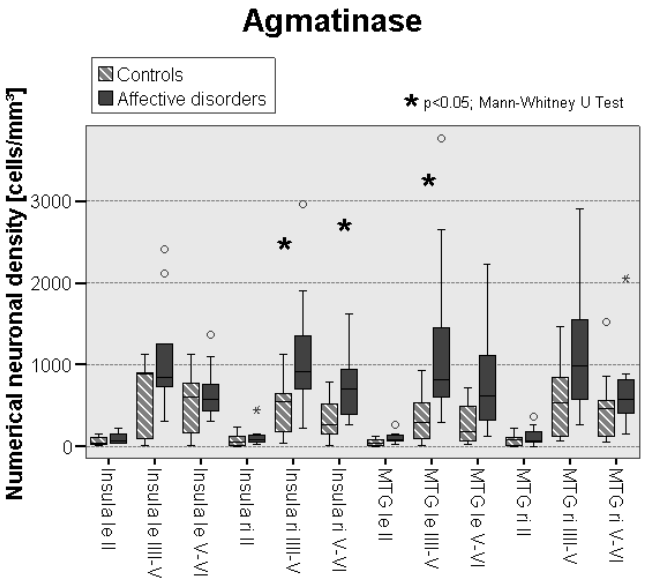

Figure 3: Numerical density of agmatinase-expressing neurons in the insular and temporal cortex of controls and depressed patients. MTG, medial temporal gyrus (of the neocortex); Le, left hemisphere; Ri, right hemisphere; I-VI, cortical layers.

3.2. HCAT1

With regard to hCAT1, multiple pyramidal and interneurons were immunoreactive for the protein, with interneurons being very intensely immunostained. (Fig. 1B) Occasioanally, hCAT1 immunoreactive axons were found. In addition, CAT1 was seen in numerous astrocytes. In patients who had suffered from a mood disorder, a significantly increased density of immunoreactive neurons was estimated in the CA2 region of the hippocampus (Fig. 4).

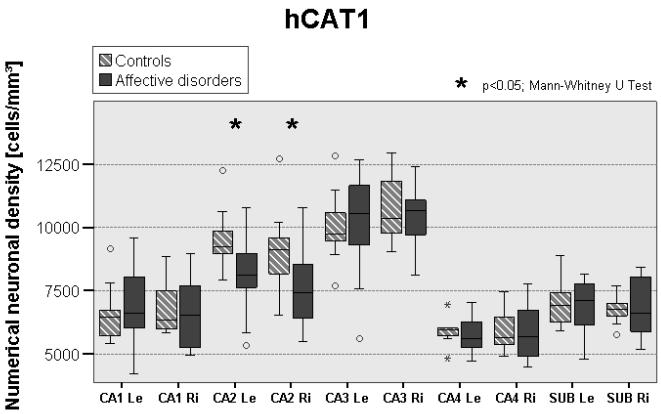

Figure 4: Numerical density of hCAT1-expressing neurons in the hippocampus of controls and depressed patients. CA1, CA2; CA3, CA4, subfields of the hippocampus; SUB, subiculum; Le, left hemisphere; Ri, right hemisphere.

4. Discussion

The enzyme agmatinase is an inactivator of the putative endogenous antidepressant agmatine (for recent considerations, see Bernstein et al., 2012). Our current findings clearly show that in depression elevated agmatinase expression is not restricted to the hippocampus, but can also be found in other brain areas. Although we currently cannot demonstrate an increase in agmatinase enzyme activity on a cellular level in depression, it can be assumed that the observed increase in protein expression is accompanied by an increased enzymatic activity. This increased activity may result in a local reduction of brain tissue agmatine levels, thus reducing "anti-depressant capacity" of the brain in depression (Bernstein et al., 2012). Hence, increased inactivation of agmatine may play a central role in the pathogenesis of the disease, and "normalizing" its brain levels by depressing agmatinase expression/ activity (by perazine-1-carboxamidine or another agmatinase inhibitor; Kitanaka et al., 2014) might be a future therapeutic option. Unexpectedly, we found an increased (not decreased) expression of hCAT1 in the hippocampus of subjects with depression. However, the up-regulation of an arginine transporter might be compensatory to improve the arginine supply of the brain. This seems to be obvious since arginine levels are known to be reduced in depression (at least in blood platelets; Pinto et al., 2012). Besides, hCAT1 has been identified as a mediator of the NMDA receptors by acting via the rapamycin-mTOR pathway (Huang et al., 2007), which is disturbed in depression (Jernigan et al., 2011). It remains, however, to be elucidated whether antidepressant medication contributes to the increase of hCAT1 expression in mood disorders.

The authors declare no conflict of interest.

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