REVIEW

Critical Care Medicine

Reduced Glutamatergic Neurotransmission as Possible Indicator of Unfavorable Prognosis

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Abstract

The paper summarizes the results of experimental and clinical studies showing a reduced function of the glutamatergic neurotransmitter system (GNS) in the development of critical states of the organism. Reduced function of GNS is considered as an unfavorable prognostic factor associated with key mechanisms of thanatogenesis. (*Int J Biomed.* 2017;7(1):15-23.)

Key Words: glutamate • glutamine • kynurenic acid • critically ill patients

Abbreviations

AAs, amino acid; **CNS**, central nervous system; **CVVH**, continuous veno-venous hemofiltration; **GNS**, glutamatergic neurotransmitter system; **GABA**, gamma-aminobutyric acid; **Glu**, glutamate; **Gln**, glutamine; **ICU**, intensive care unit; **IDO**, indoleamine 2,3- dioxygenase; **KP**, kynurenine pathway; **NMDAR**, N-methyl-D-aspartate receptor; **RGN**, reduced glutamatergic neurotransmission; **TDO**, tryptophan 2,3 dioxygenase.

Introduction

Modern methods of metabolomics research allow identifying a super-wide range of low molecular weight compounds. This presents a great potential for their use in critical care medicine for understanding the relationships between the factors of microorganisms and the course of the pathological process that is key to improving the disease outcomes.^[1] Metabolomics promotes the development of “personalized” medicine based on the application of patient-specific profiles, incorporating genetic and genomic data as well as clinical and environmental factors, to assess individual risks and to improve diagnostics and the target treatment.^[2,3]

GNS of humans and animals has a wide range of neurogenic and non-neurogenic functions, many of which are still little known. Through GNS components (Glu, Gln, its inactive metabolite and precursor, a wide range of Glu

receptors, transporters, and enzymes), transmission of nerve impulses, glutamatergic neurotransmission, is carried out. Glu is an important signaling molecule and a major excitatory neurotransmitter in GNS; its receptors are widely prevalent in phylogenetically very distant species of living organisms.^[4] Most, if not all, cells of the central nervous system (CNS) have at least one type of Glu receptor.^[5] Under normal physiological conditions, Glu is released as a neurotransmitter into the synaptic cleft and initiates the propagation of action potentials. GNS components have been also identified outside CNS: in heart, liver, kidney, lung, thyroid gland, and skin, in the enteric nervous system, in the “gut-brain axis,” in plasma and blood cells.^[6-9] GNS is involved in the functioning of multiple organ systems: CNS, cardiovascular, respiratory, gastrointestinal, and immune systems, and the hypothalamic-pituitary-thyroid axis.^[9-11] Glu-dependent activation of NMDARs in heart allows sufficient influx of calcium to increase myocardial contractility and systolic pressure.^[12] Glu induces contraction of ductus arteriosus through GluR-mediated noradrenaline production. Supplementation of glutamate might help to prevent patent ductus arteriosus in extremely preterm infants.^[13] Glu also modulates the motor function of the gastrointestinal tract.^[14]

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Over the last 4 decades, a number of studies have shown that neurons release more than one neurotransmitter. It has been suggested that monoamine and cholinergic neurons use Glu as a co-transmitter. There is evidence of co-release of Glu and GABA, excitatory and inhibitory fast neurotransmitters, from a single axon terminal in neurons.^[15-17] GNS is involved in synaptic and diffuse neurotransmission, the formation of neurons^[118] and synaptic plasticity.^[4] Glu and Gln are multifunctional AAs, which are involved in a large number of metabolic reactions aimed at the detoxification of ammonia, an increase in resistance to hypoxia, and the formation of the antioxidant glutathione, ATP, AAs, and other proteins.^[19]

Evidence of Glu participation in the regulation of physiological and pathological processes in other organs and tissues (lung, kidney, liver, heart, gastrointestinal tract, and immune system) has been obtained only in recent years. Earlier studies on the GNS role in the development of patients' critical condition were (in the vast majority) experimental or postmortem studies and were focused on the action of Glu in CNS. According to E. Aleksandrova et al.,^[21] the results of these studies are numerous debated concepts regarding the damaging effect of the increased or decreased extracellular Glu levels on the activity of brain neurons in experimental models of trauma, ischemia, and inflammation.

Glu is not only the primary excitatory neurotransmitter in the adult brain, but also a critical transmitter for signaling neurons to degenerate following stroke. Excitotoxicity, the specific type of neurotoxicity mediated by Glu, is a primary contributor of ischemic neuronal death and other cellular components of the neurovascular unit. Cerebral ischemia leads to a massive release of Glu, which stimulates NMDARs and induces calcium influx through these ionotropic receptors; the calcium-dependent activation of death-signaling proteins that are immediately downstream of the receptors triggers a plethora of signaling cascades that work synergistically to induce neuronal death.^[22,23] As scientists begin to understand the critical role of NMDARs and calcium input in excitotoxicity, several strategies have been developed against glutamate excitotoxicity; however, none of them have shown positive results in clinical practice so far, and all NMDAR antagonists failed in clinical trials.^[24]

In the case of acute processes such as stroke or traumatic brain injuries, glutamate excitotoxicity is thought to cause harm within a narrow timeframe after which the neurotransmitter reassumes its normal function. Therefore, the use of agents acting on NMDAR may have not only missed the window for therapeutic efficacy but also led to undesired side effects from prolonged receptor blockades.^[24] Nowadays, the concept of blood/brain glutamate grabbing or scavenging is well recognized as a novel and attractive protective strategy to reduce the excitotoxic effect of excess extracellular glutamate that accumulates in the brain following an ischemic stroke.^[25]

It is important to note that Glu can produce both adverse (neurotoxic) and positive (anti-ischemic) effects in cerebral ischemia. A study performed by T. Gan'shina et al.^[26] found an interaction between excitatory and inhibitory systems on the level of cerebral vessels.

Analysis of the results of a number of experimental and

clinical studies allows us to consider reduced glutamatergic neurotransmission (RGN) as a possible indicator of unfavorable prognosis of pathological conditions, as well as a factor associated with the development of key mechanisms of thanatogenesis^[27] and main pathological processes with self-dependent thanatological value: systemic hypoxia, sepsis, and acute kidney injury (Figure 1).

Fig. 1. Key mechanisms of thanatogenesis in critically ill patients according to G. Ryabov (1988) and the schema fragment of pathogenesis and thanatogenesis by I. Timofeev (2016)

UD - underlying disease; CUD- complication of underlying disease; CC- critical condition; TC - terminal condition; CD - clinical death; BD - biological death

The neurochemical criteria of GNS hypofunction

The main neurochemical criteria indicating hypofunction of GNS can be: 1) low level of Glu (or Glu+Gln) in the plasma and investigated tissues in comparison with reference values; 2) an increased level of kynurenic acid (KYNA), a neuroactive metabolite of tryptophan catabolism in enzymatic cascade, known as KP.

Currently, Glu concentrations in cerebrospinal fluid (CSF) and blood plasma are readily available criteria for indirect evaluation of Glu homeostasis in the body.^[8] These available biofluids are interpreted as an average representation of the surrounding tissue.^[29] The content of mediators and products of their inactivation in plasma and CSF depends on the circulation of neurotransmitters in the tissues and reflects the intensity of neurotransmitter processes.^[30]

Maintaining a relatively constant concentration of extracellular AA pool is one of the functions of the interorgan AA exchange.^[31] According to B.Bein and A. Ezhova,^[32] the constant maintenance of a certain amount of free AAs in the blood is carried out due to transfer from the gastrointestinal tract and tissue during protein decay, as well as redistribution and consumption of AAs in organs and tissues. The exact mechanisms that lead to a change in the plasma Glu levels remain unknown, but in general, it is release and redistribution of Glu between organs or the activation of neutralizing natural mechanisms.^[33] From CNS, neurotransmitters and their metabolic products are released in the blood and CSF.^[29] The diffuse and synaptic types of neurotransmission have complex mechanisms of interaction. Researchers have described the movement of Glu from synoptic slit and its participation in the diffuse type of neurotransmission, as well as interactions of “diffuse” Glu with the synaptic (presynaptic and postsynaptic) receptors.^[34] For regulation of extracellular concentrations of Glu, its transporters also have a significant effect. Glu transfer by transporters is carried out in both directions of the cell membrane, depending on the gradient of the ions Na⁺/K⁺ and amino acid itself and may change as a result of metabolic processes. Another major source of extracellular Glu is its release by neurons and glial cells through exocytosis. This process can be triggered by activation of glial glutamate receptors and represents Ca²⁺-dependent process.^[34]

It should be noted that Glu level is closely associated with plasma concentration of KYNA, which is one of the end products of tryptophan formed in KP. KYNA in supraphysiological concentrations (micromolar levels) has an antagonistic effect on all three ionotropic Glu receptors and $\alpha 7$ nAChR.^[35-37] An increase in KYNA concentration is accompanied by a decrease in Glu release, and a decrease in extracellular levels of dopamine^[38] (Glu via ionotropic receptors indirectly stimulates the dopamine release).^[39]

The modern methods of metabolomic research are characterized by high manufacturability analysis and, accordingly, the degree and accuracy of determination of low molecular weight substances.^[30] Among the analytical methods used in metabolomics research, liquid chromatography/mass spectrometry (LC/MS) has been shown to be one of the best techniques in terms of selectivity, sensitivity, and reproducibility.^[40,41] It provides the highest level of metabolite coverage, using a unified analytical technique. In LC-MS, limit of sensitivity is about 5nM to 10nM and the number of detectable characteristics - from 5000 to 20000.

In magnetic resonance spectroscopy (MRS), where the detection limit is usually 5 μ M to 10 μ M, a number of recognizable compounds are from 40 to 200, depending on the biological material to be analyzed.^[42] Measuring the Glu concentration by MRS with the usual values of the magnetic field causes difficulty in distinguishing between Glu and Gln; for this reason, a composite index is often used.^[43] Another aspect of modern metabolomic research is the application of multivariate statistical analysis^[40] and the construction of predictive models.^[30]

Thus, the levels of metabolites in the blood plasma reflect the systemic reactions of the body and are considered as a body's response to the disease.^[44,45] As will be presented later, with hypofunction of GNS, likelihood of an unfavorable outcome of the pathological process in the following period of time increases, and the development of each thanatogenesis mechanism is associated with a reduced functioning of GNS.

RGN as a risk factor for an unfavorable course of the pathological processes in critically ill patients

According to M. Poeze et al.^[46] and T. Hirose et al.^[47], relatively lower values in plasma Glu concentrations may be an independent predictor of poor outcome in patients with sepsis. E.V. Alexandrova^[21] found that the syndrome of RGN is prognostically less favorable than glutamatergic redundancy in patients with severe traumatic brain injury.

In our studies, the risk of an adverse outcome in the 28-day period in ICU was significantly higher (5 times) in critically ill patients (with different underlying pathology) with initially reduced plasma Glu levels than in patients with baseline Glu within reference levels. Prognostic significance of positive testing for reduced plasma Glu level in critically ill patients was 82%, and specificity - 84%.^[48,49] The 28-day survival rates were less in critically ill patients with reduced plasma Glu levels compared to reference values (Gehan's Generalized Wilcoxon test, $P=0.01544$; Cox's F-test, $P=0.00163$; Cox-Mantel test, $P=0.00243$; Peto & Peto's and Prentice's generalized Wilcoxon, $P=0.00738$; the logrank test, $P=0.00507$). We found direct correlations between reduced plasma Glu levels and the Apache II Score and the SOFA scores for the cardiovascular, hepatic, coagulation, renal and neurological systems (Table 1); and we identified inverse correlations between the reduced plasma Glu levels and hemoglobin oxygen saturation in the superior vena cava (ScvO₂).

Table 1.

Correlations between reduced plasma Glu levels and the Apache II Score and the SOFA scores

Reduced plasma Glu level	Gamma correlations ($P < 0.05$)					
	SOFA (total score) $r = 0.564$	SOFA scores				
		Cardiovascular system $r = 0.614$	CNS $r = 0.542$	Liver $r = 0.569$	Kidneys $r = 0.583$	Coagulation $r = 0.362$
	APACHE II score $r = 0.400$	Plasma procalcitonin level $r = 0.368$	Plasma lactate level $r = 0.373$	S _{cv} O ₂ $r = 0.621$		

The impact of the reduced plasma Glu level on 28-day survival rate of critically ill patients was shown in regression models built using the software package Statistica 12 (adequate models: Cox's proportional hazards model, $P=0.00619$, exponential regression, $P=0.00323$; lognormal regression, $P=0.01944$; normal regression, $P=0.00524$) and Predictor Screening on basis of Statistica Data Miner Recipes (Chi-square=6.946742, $P=0.0084$).^[48; 49]

Currently, in contrast to previous studies, H. Buter et al.^[50] have shown an association between plasma Gln levels and severity of clinical condition calculated on the APACHE-IV scale. According to Y. Lin et al.,^[51] Gln reduces apoptosis of cardiomyocytes and increases their functional activity at low pH. Under experimental conditions, Gln increased the lifespan of rats after asystole.

The prognostic role of the high KYNA content in patients with unfavorable outcome has been confirmed in several clinical trials. In study by W. Dabrowski et al.,^[52] the concentration of KYNA in the plasma of septic patients with good clinical outcomes decreased gradually over the course of CVVH. In contrast, the concentration of KYNA in the plasma of septic patients with poor clinical results did not decrease over the course of CVVH. In fact, an increase in KYNA concentration was observed. At the same time, the concentrations of CRP, PCT and lactate decreased during CVVH in this group of patients. In the study of G. Ristagno et al.,^[53] which included 245 patients resuscitated in the first day after cardiac arrest, high KYNA levels were independently associated with ICU death and with 12-month death. L. Darligton et al.^[54] showed that KYNA levels were significantly raised in patients with acute stroke who died within 21 days compared with those who survived; KYNA levels were significantly higher at all study time points (the first, second, third, fourth and seventh days after the stroke) in this group of patients.

RGN and hypoxia

In critically ill patients with signs of systemic hypoxia, reduced levels of plasma Glu concentrations were observed 5 times more often ($P=0.019478$, $r=0.833$) than in critically ill patients without systemic hypoxia. Direct correlation was found between reduced plasma Glu content and each criterion of systemic hypoxia: an increased level of lactate in the blood plasma ($r=0.710$) and reduced ScvO₂ level ($r=0.621$). The relationship between the formation of systemic hypoxia and reduced Glu content in the blood plasma was shown in the logistic regression model built using Predictor Screening on basis of Data Miner Recipes Data Miner (Statistica 12).^[55]

The above data of clinical trials are consistent with the results of experimental work. Changes in the metabolism of Glu and Gln in hypoxic conditions have been presented by several authors as a phenomenon of "metabolic reprogramming". The increased consumption of Glu and/or Gln in hypoxia is shown as a tissue adaptation to anaerobic conditions. Glu has been described as a more "preferred" substrate for fatty acid synthesis than Gln.^[56-60] In a pilot study by H. Baran et al.,^[61] an increase in KYNA level had a direct correlation with the severity of hypoxia. KYNA levels in tissues increased by 44%

after 5min of asphyxia and 302% after 20min of asphyxia (the critical time limit of survival). According to G. Ceresoli-Borroni and R. Schwarcz,^[63] up to 6 h, asphyxia caused 160-267% increases in KYNA levels in neonatal rats. Changes in the metabolism of Glu, Gln and KYNA in the body during hypoxia, which were identified in the experimental and clinical studies, are shown in Figure 2.

Fig. 2. Changes in the metabolism of Glu and Gln in hypoxic conditions

RGN and acute kidney injury

In a study conducted by H. Chua et al.,^[64] critically ill patients with acute kidney injury (AKI) in more than 50% of cases had plasma Glu levels below the reference value. In our early study,^[65] we found more frequent reduction in levels of Glu and Gln in the plasma of critically ill patients with AKI compared to critically ill patients without AKI. Frequency Glu reduction in plasma increased with the increasing severity of AKI: 50% for AKI-I and 73% for AKI-III ($r=0.481$; $P=0.03$). Relationships between the AKI development and reduced plasma Glu level, as well as with the degree of AKI severity, were shown in models of logistic regression and artificial neural networks. These data are consistent with the results of studies carried out on animals. In an experimental study performed by M. Duran et al.,^[66] after the initiation of acute renal failure, which was modeled experimentally in two ways (ischemic and chemical forms), reduced Glu levels in the renal cortex and plasma were found in both cases. According to R. Goldstein et al.,^[67] significantly decreased plasma Glu concentrations were found in cats with various stages of chronic renal failure. In experimental study of I. Montañés et al.,^[68] the cortical concentrations of glutamine and glutamate in dogs were lower

in the recovery phase (48 hours) after acute renal ischemia than in control kidneys. Changes in the metabolism of Glu, Gln and KYNA in the body during AKI, which were identified in the experimental and clinical studies, are shown in Figure 3.

KYNA is a known uremic toxin.^[69] Its content in patients with uremia exceeds the reference values by many times and correlates with the development of uremic symptoms. Toxic effects of the relevant concentrations of KYNA have been confirmed by experimental studies.^[70] In the study of D. Pawlak et al.,^[71] in spite of haemodialysis, plasma KYNA concentration was elevated in uremic patients in comparison with healthy volunteers. The high concentrations of KYNA positively correlated with degree of the renal insufficiency in rats with experimental chronic renal failure.^[72]

Fig. 3. Changes in the metabolism of Glu, Gln and KYNA in AKI

RGN and sepsis

Septic shock is a major cause of death in critically ill patients who are treated in ICUs. In patients with sepsis, according to R. Langley et al.,^[73] the levels of Glu and Gln at enrollment to ICU and 24 hours later after treatment were significantly lower than in uninfected patients. Similar results were obtained in the study of W. Mickiewicz et al.,^[74] thus, in the first 24 hours of admission to ICU, the plasma Glu concentration in patients with septic shock was lower than that of ICU patients with the systemic inflammatory response syndrome but not suspected of having an infection ($P=0.00048$).

Our studies were carried out in 2013–2015 independently and simultaneously with foreign studies. In our studies and studies of foreign researchers, the “classical” diagnostic criteria for sepsis were applied (without revisions adopted in 2016). To enable comparison, all data are presented in the

original version. According to our results,^[75] the decreased plasma Glu level was observed 9.8 times more frequently in ICU patients with sepsis compared to ICU patients without criteria for sepsis ($P=0.00028$, ML chi-square test; $r=0.890$, Gamma statistic). With increasing severity of septic process (from severe sepsis to septic shock), frequency of the decreased plasma levels of Glu and Gln increased by 2 and 4 times, respectively ($P=0.0208$, ML chi-square test; $r=0.625$, $r=0.730$, Gamma statistic). The causal link between the reduced Glu levels and the development of sepsis, as well as its severity, in ICU patients was shown in statistical models.

Clinical findings are consistent with experimental results. According to C. Boutry et al.,^[76] with experimental endotoxemia almost all circulating AAs, including Glu, decreased. A supplementation with 4% monosodium glutamate (MSG) or an isomolar amount of glutamine failed to restore Glu concentrations in plasma and muscle. A significant reduction in the concentration of extracellular Glu was determined during progressive inflammatory reaction induced by administration of LPS.^[77] E.V. Sabadash and S.N. Skornyakova,^[78] in a study on animals, showed a progressive decrease in the plasma Glu concentrations with an increase in the severity of infection.

H. Buter et al.^[50] described a correlation between pre-operative plasma glutamine levels and the presence of a positive culture after cardiac surgery. In another study,^[79] researchers also found that plasma Glu levels were determined by the severity of illness and the presence of an infection in ICU patients.

A considerable amount of evidence has accumulated as concerns interactions between KP and immune dysregulation in the development of septic shock. KYNA is one of the end products of tryptophan formed in KP. In the first step of this process, tryptophan is oxygenized by TDO or IDO into kynurenine, which is then transformed by kynurenine aminotransferases into KYNA.

Physiological concentration of the human plasma KYNA ranges between 25nM and 60nM.^[80, 81] IDO occupies a key position connecting the immune system and KP.^[82, 83] As is well known, IDO is rate-limiting enzyme of tryptophan catabolism and plays a pivotal role in immune tolerance.

P.Tattevin et al.^[84] showed that IDO activity gradually increased according to sepsis severity, and septic patients who died had higher IDO activity on admission than did survivors ($P=0.013$). IDO activity was markedly increased in patients with septic shock (0.235 [IQR, 0.152–0.481], +751%, $P<0.001$), in patients with severe sepsis (0.123 [IQR, 0.068–0.271], +344%, $P<0.001$), and in patients with sepsis (0.033 [IQR, 0.031–0.052], +20%, $P=0.008$), as compared with control participants (0.028 [IQR, 0.025–0.036]). In addition, IDO activity was correlated with SAPS II score and day LOD score. As was shown, increasing plasma KYNA concentration might predispose to sepsis and septic shock in patients after multi trauma and its level is related to severity of infection.
[52, 85, 86]

Changes in the metabolism of Glu, Gln and KYNA in the body during sepsis, which were identified in the experimental and clinical studies, are shown in Figure 4.

Fig. 4. Changes in the metabolism of Glu, Gln and KYNA in the body during sepsis.

Factors of the “generalized” hypoaminoacidemia and increased KYNA content in the development of a patient’s critical condition

Factors of the “generalized” hypoaminoacidemia in the development of a patient’s critical condition are the increased AA distribution due to vasodilation and increased permeability of the endothelium, an inhibition of the synthesis of a number of AAs in the liver and their increased consumption in central tissues (immune system, liver, spleen, wound). [46, 87]

Experimental data have shown that stress factors increased the KYNA formation and other metabolites of KP of tryptophan catabolism by activating the secretion of glucocorticoids and a significant increase in the activity of TDO^[88] and IDO, which is regulated by cytokines.^[89] The increased KYNA concentration after acute physiological stress was observed in clinical studies performed by E. Kotlinska-Hasiec et al.^[90]

Conclusion

There are many factors that can influence the course of the pathological process in critically ill patients. One of them, apparently, is RGN. In this review article, we have attempted to summarize the currently available evidence on the connection between low Glu content and a high KYNA level with each of the key mechanisms of thanatogenesis and unfavorable outcome in critically ill patients. The mechanisms behind the association of low plasma Gln levels and low Glu levels with severity of illness or mortality in critical illness

are not fully understood.^[91,92] Whether plasma glutamine and glutamate levels can be used to identify critically ill patients with poor prognosis needs further study.

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