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Metabolic Dysfunction and Relationship in Human Frontoparietal Cortex in Severe Traumatic Brain Injury: Single-Voxel ^{1}H Magnetic Resonance Spectroscopy Study

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Abstract—^{1}H-magnetic resonance spectroscopy revealed that apparently normal (from the data of magnetic resonance imaging) human brain frontoparietal cortex in the subacute stage of traumatic brain injury is characterized by decreased level of N-acetylaspartate (NAA) and increase in levels of myoinositol, choline-containing compounds (Cho), and creatine/phosphocreatine (Cr). Correlations between Cr, Cho, and NAA were established. We propose a scheme of neuronal metabolic processes that joins these substances.

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Key words: magnetic resonance spectroscopy, severe traumatic brain injury, frontoparietal cortex

The functioning of metabolic circuits in living brain that supports biological functions of the central nervous system (CNS) is one of the least studied problems. Magnetic resonance spectroscopy (MRS) is a noninvasive method allowing investigation of biochemical processes in organs and tissues *in vivo*. Modern medical magnetic resonance imaging scanners (field strength of 3 T) make it possible to record well-resolved spectra of metabolites in small local volumes of brain tissue *in vivo* and determine concentrations of these substances during the dynamics of metabolic processes. Changes in metabolite concentrations serve as vital indicators of violations of the most important metabolic pathways in various brain structures in pathological states and provide information on peculiarities of neuronal and glial cell metabolism in normality and pathology [1, 2].

The ^{1}H magnetic resonance spectra of normal human brain contain peaks corresponding to methyl groups of choline-containing compounds (Cho; chemical shift $\delta = 3.2$ ppm) and phosphocreatine/creatine (Cr; $\delta = 3.0$ ppm), *N*-acetyl group of *N*-acetylaspartate (NAA; $\delta = 2.0$ ppm), protons 1, 3, 4, and 6 of the inositol ring of myoinositol (mI; $\delta = 3.56$ ppm), and glutamine/glutamate (Glx; α-CH, $\delta = 3.75$ ppm) [1-3].

Metabolism and the role performed by these substances in cells and tissues are known for most of them. The main function of the creatine kinase system, which includes creatine and phosphocreatine, is to maintain ATP level in cells. Glutamate is an excitatory neurotransmitter, and glutamine is its precursor and the product of glutamate transamination. Cho and mI are involved in lipid metabolism. Myoinositol is also implicated in processes that include inositol-polyphosphate second messengers [3]. The signal from mI is used as a noninvasive marker of astrocytes [3, 4]. The role of NAA is insufficiently understood. It was established that, in measurable amounts, NAA is only present in neurons and that the

Abbreviations: Cho, choline-containing compounds; Cr, creatine/phosphocreatine; DAI, diffuse axonal injury; GCS, Glasgow coma scale; Glx, glutamine/glutamate; Lac, lactate; mI, myoinositol; MRS, magnetic resonance spectroscopy; MRI, magnetic resonance imaging; NAA, *N*-acetylaspartate; PCho, phosphocholine; SAM, S-adenosylmethionine; TBI, traumatic brain injury.

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concentration of NAA is in direct proportion to the level of fully functional neurons. Therefore, the signal from NAA in brain spectra *in vivo* can be used as a marker of neuronal integrity and viability [5].

Quantification of ^{1}H MRS visible metabolites in various human brain structures is of exceptional value for diagnosis and prognosis of CNS diseases, because many of these diseases are characterized by high intra-population variability of clinical symptoms in the course of disease development. Since reliable biological markers are still absent for most of these diseases, the data of magnetic resonance spectroscopy provide direct diagnostic criteria of pathology [5-7]. Moreover, the possibility of intravital and simultaneous determination of concentrations of the mentioned metabolites in operating cerebral structure opens avenues to studies biochemical processes *in vivo* that are still poorly understood.

Analysis of proton spectra of human brain in traumatic brain injury (TBI) is primarily focused on measurement of the neuronal marker NAA and retrieval of significant prognostic markers of TBI severity and outcome. A correlation was found between the trauma outcome and NAA/Cr ratio in the splenium of patients with different TBI outcome indices on the Glasgow coma scale (GCS) [8]. In particular, a correlation was found between the NAA level in normal appearing, according to magnetic resonance imaging (MRI), frontal and occipital lobes and the level of consciousness on the Glasgow coma scale [9]. The review of ^{1}H MRS studies of different brain structures in children reported before 2006 indicated that neurological and cognitive deficits resulting from TBI correlate with decrease of NAA and accumulation of Cho [10]. In mild TBI (14 points on the GCS) total NAA concentration in the brain decreases by 12% [11, 12]. In severe TBI the level of NAA decreases and Cho increases in apparently intact (according to MRI) white matter [13], which, according to the authors, is indicative of diffuse axonal injury (DAI). In another report, the authors did not find significant alterations in Cho and Cr levels in white matter in TBIs of different severity [8]. According to [14], decreased level of NAA in white matter reflects the loss of neuronal function due to DAI. It was shown [15] that decrease in NAA/Cr ratio in apparently intact (from the data of MRI) cerebral cortex of children correlated with severity of TBI. In review [16], the data of a series of studies are presented demonstrating decrease in NAA level in gray matter of occipital lobes in the acute period of TBI. According to [17], in supraventricular brain tissue of patients with mild TBI the Glx level is decreased in gray matter, while the Cr level is increased in white matter. Elevation of Glx level was found in occipital lobes of children with TBI; nevertheless, none of the spectral parameters (NAA, Cho, Cr, mI, and Glx) was associated with outcome [18]. The same authors reported that elevated level of mI in occipital brain lobes of children within the acute period of severe TBI is indicative of unfavorable outcome of the injury [19]. In patients with secondary cortical atrophy caused by severe or moderate TBI, the level of NAA is decreased in corpus callosum due, in the authors' opinion, to axonal death [20]. The signal of lactate (Lac) in injured brain, whose appearance was repeatedly reported, suggests activation of anaerobic cerebral metabolism of glucose at the early posttraumatic stage; in the subacute period the Lac signal appears because of development of inflammation [16, 23]. According to [21], presence of Lac is indicative of unfavorable outcome of posttraumatic brain injury. According to [22], decrease in NAA and elevation of Lac contents in acute and subacute TBI periods in children correlates with rating of neurological deficit in the late period of posttraumatic brain disease.

Experimental models of TBI make it possible to compare the data of MRS with those obtained by invasive methods and reveal cellular mechanisms of brain injury in trauma. While decrease in NAA, Cr, Cho, mI, and glutamate levels immediately after TBI in the zone of perifocal edema in rat brain reflects nervous tissue injury found by histological analysis, the elevation of Cho and mI levels on the seventh day after TBI is caused by activation of glia and proliferation of glial cells, and elevation of NAA level by restoration of mitochondrial and neuronal functions [24]. The signal of Lac appears in spectra of injured brain tissue and achieves a maximum on the seventh day after TBI, which, in author's opinion [24], is associated with inflammatory processes and activation of glia. Astroglia is moderately activated in the contralateral volume of the intact hemisphere, although spectral alterations are not found [24].

Thus, according to the majority of studies, TBI causes decrease in NAA level in different brain structures, which are apparently intact according to diagnostic MRI. This decrease in white matter is a symptom of DAI. The data obtained on rats [24, 25] suggest that a reversible decrease in NAA level in TBI results from reversible disturbance of ATP synthesis in mitochondria of neurons. The data on the effect of TBI on the level of Cho are contradictory; Cr, mI, Glx, and Lac in TBI are insufficiently understood.

Signals of NAA, Cr, and Cho, which are present in all spectra of normal appearing brain tissue regardless the method of spectral recording (long or short echo time), are considered either as cell markers or as markers of individual unrelated metabolic pathways. We have shown that in cerebral cortex of healthy humans concentrations of these metabolites are interdependent, and we analyzed the character of these dependencies [26]. It is important to determine whether the relation between NAA, Cr, and Cho existing in apparently intact (according to diagnostic MRI) cortex remains in TBI.

The aim of this work was to analyze the proton spectra of normal (from the data of MRI) frontoparietal cortex of children with severe TBI, reveal interrelations between the levels of metabolites, and propose a scheme of metabolic processes that satisfies the observed effects.

METHODS OF INVESTIGATION

We studied a group of 34 children of ages ranging from 5 to 16 years (average age 12.7 years). The control group consisted of 16 children. The two groups had similar age and sex distributions. The group with TBI consisted of 18 patients with severe brain injury, in which, according to MRI, total volume of injured tissue was 30-50 ml; the lesions contained compressed tissue and hematoma.

MRS examination was carried out in the subacute period of trauma (15-30 days after TBI). Studies were performed using a Phillips Achieva 3.0T medical tomograph with a magnetic field of 3 T equipped with built-in ViewForum software package. Diagnostic MRI was carried out according to a standard protocol to obtain the axial T2-weighted, T2-FLAIR, sagittal T2-FLAIR, and coronal T1-FLAIR images, as well as native MRI angiography in three projections to exclude vessels from the volume of interest during MRS. After obtaining diagnostic MRI images, the spectroscopic voxel was oriented at intact gray matter of frontoparietal cortex. This structure is optimal for studies of metabolism of injured cortex area because frontoparietal lobe lacks the counterattack mechanism of injury. The volume of interest was assigned using the PRESS pulse sequence with echo time TE = 35 msec and time delay between repeated pulses TR = 2000 msec. The voxel volume was V = 3 cm^3. The magnetic field homogenization (shimming) was carried out automatically in a cube, 25-mm on each side; the center of the cube coincided with the center of the spectroscopic voxel. The signal from water was attenuated with a presaturation pulse. To obtain the optimal signal-to-noise ratio for a minimal time of study, we used signal accumulation number of 32.

The free induction decay signal was processed by the built-in SpectroView software package with automatic narrowing of lines with parameters 1.5 for the exponential factor and 3 for the Gaussian curve. The baseline was adjusted automatically and completed manually. Metabolite signals were approximated with Gaussian lines, and then their amplitudes were calculated; the amplitude of each signal was corrected for the resonance amplitude of the unsuppressed water. Since the content of water in apparently intact (according to MRI data) tissue is constant, it was taken as an internal concentration standard [27]. The spectral data were statistically processed using the Statistica 6.0 software package. Compliance of obtained value distributions with the normal one was estimated by apposition of an empiric normal distribution curve on frequency histograms. Significance of difference between the mean values was determined using the Student's *t*-test with the statistical significance level $p \leq 0.05$. The relationships between the concentrations of different metabolites were revealed using Pearson correlation at p level ≤ 0.05.

RESULTS AND DISCUSSION

Figure 1 displays typical spectra of frontoparietal cortex of a healthy child (a) and undamaged frontoparietal cortex of a patient with TBI (b). One can see that the NAA signal in spectrum (b) is considerably less intense in relation to other signals than in spectrum (a). Statistical analysis of the spectral data suggests that in apparently undamaged (according to MRI) frontoparietal cortex of patients with TBI the level of NAA decreases significantly, while the levels of Cho, mI, and Cr increase (Fig. 2). The level of Glx does not differ from normal. The level of Lac was not significantly increased. Effects of TBI we have found in non-injured cortex area, namely decrease of NAA and increase in Cho levels, are in agreement with the data reported in [10]. Increase in mI level was earlier reported in undamaged white matter in the acute period of TBI [18]. The absent Lac signal and normal Glx level are comparable to the data of studies on undamaged rat brain hemisphere [24].

A previously unknown result was obtained from the correlation analysis of spectral data, which demonstrated a direct proportion between NAA, Cr, and Cho in frontoparietal cortex (table): the more NAA there is, the greater are the Cr and Cho levels. And, in turn, the less the NAA level is, the less are the Cr and Cho levels. The levels of Cr and Cho are also in direct proportion. Thus, metabolic processes involving NAA, Cr, and Cho that occur in the subacute phase of posttraumatic period in apparently undamaged (according to MRI) frontoparietal cortex are interrelated.

Comparison of the mean values of NAA and Cr indicates that the diagnostically significant change in the NAA/Cr ratio in apparently undamaged (according to MRI) cortex in injury [8, 9, 13, 15] can result from either decrease in NAA level or increase in the level of Cr. Elevation of Cr level can result from either elevated influx of creatine synthesized in kidney, pancreas, and liver or activation of its synthesis in brain cells. Creatine is synthesized in two stages with implication of L-arginine-glycine amidinotransferase (AGAT; EC 2.1.4.1) and guanidine acetate-N-methyl transferase (EC 2.1.1.2). The genes encoding both enzymes are abundantly expressed in both neuronal and glial cells [28]. The

Statistically significant ($p < 0.005$) linear correlation coefficients (R) between metabolites in intact human brain gray matter and undamaged gray matter in TBI

	R_{NAA-Cr}	$R_{NAA-Cho}$	R_{Cr-Cho}
Normality	0.65	0.64	0.61
Injury	0.82	0.53	0.66

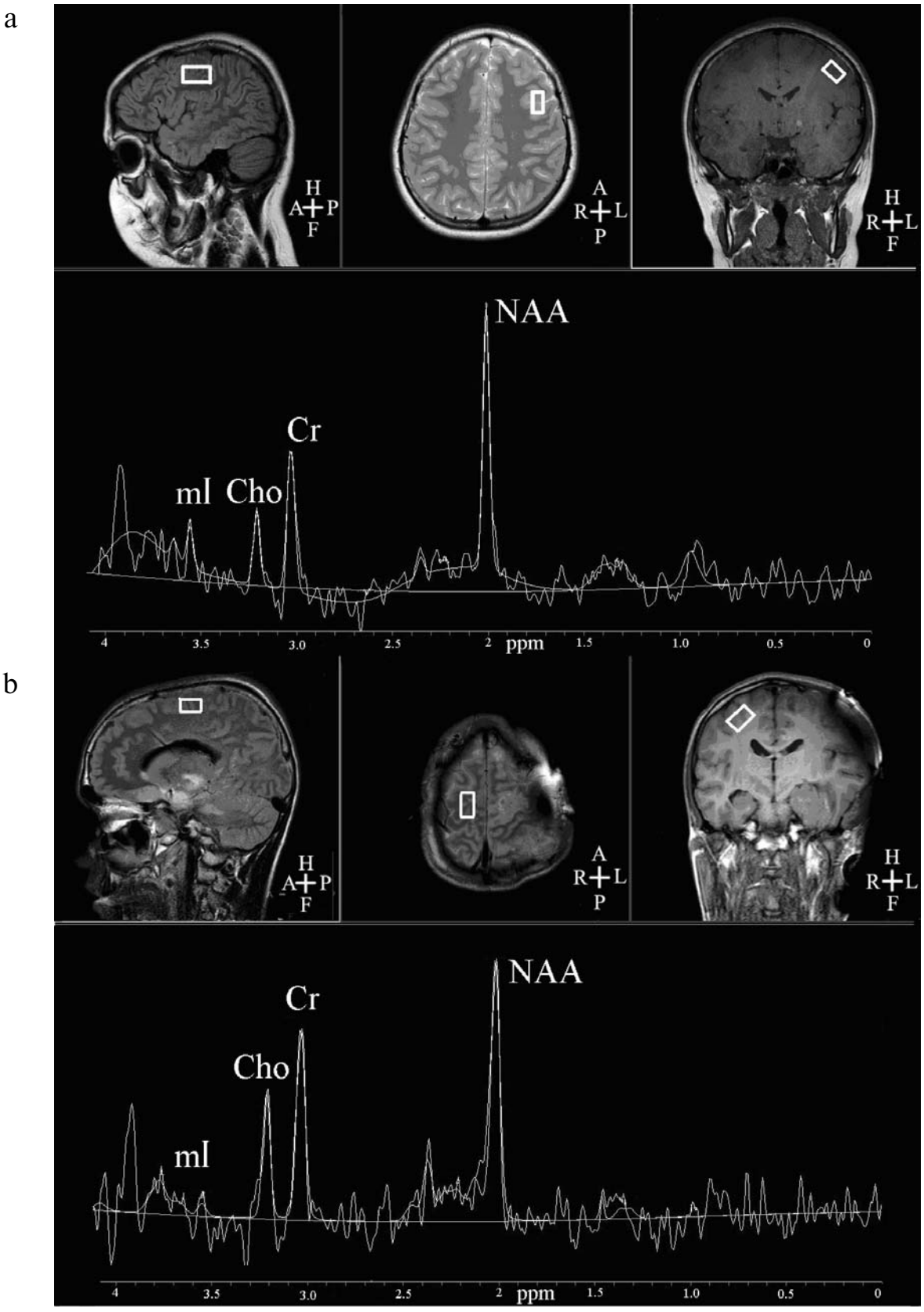

Fig. 1. ^{1}H magnetic resonance spectra of undamaged frontoparietal cortex (voxel position is shown on MRI images) in normality (a) and severe TBI (b).

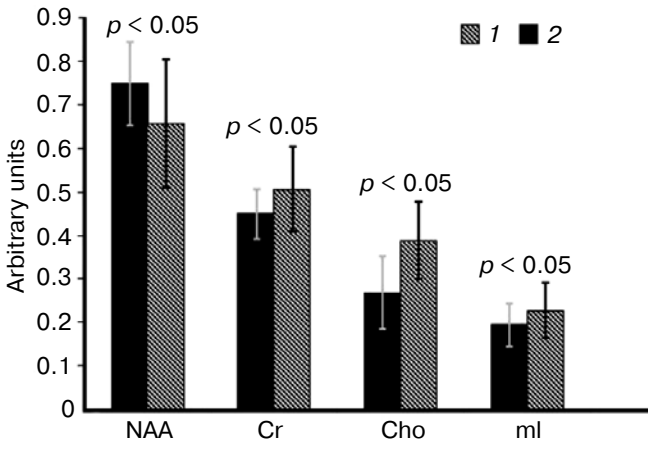

Fig. 2. Mean values (± standard deviations) of relative intensities of metabolite signals in spectra of undamaged frontoparietal cortex in normality (*1*) and severe TBI (*2*).

authors suggest that most of the cerebral creatine is endogenous. Given these data, we suppose that the level of Cr determined in the presented work undergoes elevation due to activation of creatine synthesis in brain.

Elevation of Cho signal intensity that we also found in this study probably results from activation of membrane phospholipid synthesis rather than destruction of cellular membranes. In fact, the Cho signal is a superposition of resonances from choline, phosphocholine (PCho), and glycerophosphocholine. The main input in the signal belongs to PCho. Degradation of membrane phospholipids not only elevates their concentration, but also changes the spin–spin relaxation of tissue water protons, which is well-detected on T2-weighted images in MRI. We oriented the spectroscopic voxel at a tissue characterized by unchanged parameters of water relaxation. So, we surmise that elevation of the Cho signal intensity is a result of activation of membrane phospholipid synthesis. The source providing elevation of the Cho

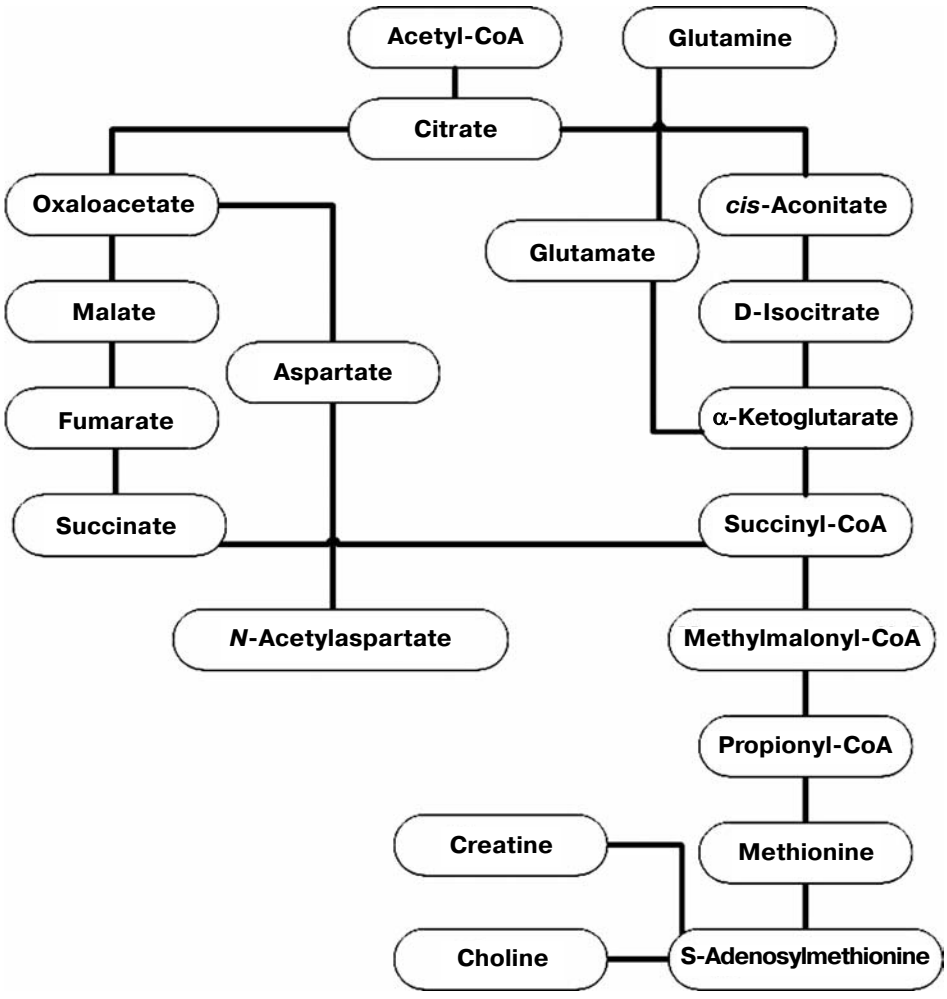

Fig. 3. Scheme of metabolic circuit connecting Cr, Cho, and NAA.

pool can be PCho, which – in brain, particularly neurons – is synthesized in three successive *trans*-methylation reactions from phosphatidylethanolamine [29, 30].

Increase in intensity of the mI signal is probably associated with activation of astroglia. The growth of mI level determined by gliolysis and astroglia activation was found [24] in tissue immediately adjacent to the focus of experimental injury. High mI level in occipital lobe white matter in the acute period of severe TBI in children seems to be caused by gliolysis [18].

Thus, elevation of Cr, Cho, and mI levels suggests activation of energy-dependent synthesis in apparently undamaged (according to MRI) cerebral cortex of children in subacute TBI. The level of NAA decreases simultaneously. Reversible decrease of this signal in TBI can be associated with energy metabolism disorder caused by ischemic effect of injury [25], as well as with acute stress reaction of neurons [24]. The latter characterizes the early period of TBI, while our study was performed in the subacute period. In ^{1}H spectra of brain the signs of energy metabolism disorder due to oxygen and glucose deficiency are the presence of Lac signal and decrease in Glx signal [24], which we did not observe in this work. So, the suspicion arises that the decrease in NAA level in undamaged frontoparietal cortex in subacute TBI can result not only from mitochondrial dysfunction, but also from activation of energy-dependent synthesis of creatine and Cho. Decrease in NAA level was revealed in human visual cortex in response to neurostimulation, another energy-intensive process [31].

Correlations between Cr, Cho, and neuronal marker NAA suggest that the neuronal metabolic circuit is common for all these metabolites. Earlier, we found similar correlations in frontoparietal cortex of healthy patients [26]. The link between NAA, Cr, and Cho can be realized via the sequence of chemical reactions presented in the scheme (Fig. 3). According to this scheme, S-adenosylmethionine (SAM) is the common source of methyl groups for syntheses of creatine and PCho. SAM is connected with NAA via the neuronal Krebs cycle, which the SAM precursor methionine [32] and the NAA precursor aspartate [33] enter via succinyl-CoA and malate, respectively.

Identical correlation between metabolites in cerebral cortex of healthy persons and apparently normal (according to MRI) cerebral cortex in TBI suggests that the metabolic circuit common for NAA, Cr, and Cho is maintained in frontoparietal cortex in TBI, and factors causing changes in their mean values do not affect integrity of the circuit. Basic fibroblast growth factor may be one of these factors. It is synthesized not only by astrocytes, but also by neurons, and it has a positive effect on neuronal survival and plasticity [34]. Elevated expression of this factor in thalamus (a structure containing neurons) is observed for 28 days after TBI [24]. Increase in mean values of Cr and Cho and decrease in NAA in this work is probably caused by the fibroblast growth factor-induced activation of membrane phospholipids and their precursor PCho.

Intensities of spectral signals were in direct proportion to concentrations of corresponding metabolites in cytosol, hence the found correlations characterize a relationship between the concentrations of NAA, Cr, and PCho in the cytosol of neurons.

Thus, the proposed scheme should describe processes occurring in the neuronal cytosol and explain changes in the levels of NAA, Cr, and Cho in TBI. Increase in levels of Cr and Cho and simultaneous decrease in level of NAA in severe TBI may result from activation of the metabolic pathway of creatine synthesis in parallel with activation of synthesis of membrane phospholipids and their precursor PCho (see Fig. 3), because methionine is a precursor of both of these metabolites. Efflux of methionine decreases activity the Krebs cycle and, hence, suppresses synthesis of NAA, which likely results in decrease in its level.

Thus, correlation between Cr and Cho, NAA and Cr, and NAA and Cho can reflect a relationship between lipid energy metabolism and activity of the Krebs cycle in neurons of undamaged (according to MRI) cerebral cortex of children. Both Cr and Cho are synthesized in cytosol, whereas NAA is synthesized in mitochondria, and correlation between these levels suggests conformity between energy, lipid, and amino acid metabolism, suggesting the implication of mitochondria in regulation of neuronal metabolism *in vivo*.

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