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THE ROLE OF THE NEUROTROPHIC FACTOR BDNF IN PERINATAL HYPOXIC-ISCHEMIC ENCEPHALOPATHY

SUMMARY

In this study we determined the brain-derived neurotrophic factor (BDNF) in the serum of children with various levels of perinatal hypoxic-ischemic encephalopathy (PHIE) in acute and retrieval periods.

A higher level of BDNF is associated to a better protection of brain against destruction, maintaining the neuronal survival and differentiation. The low BDNF level in serum of new-borns with PHIE constitutes an objective criterion in prognostic of neuro-psychical and motor disabilities.

In severe PHIE the processes of neurogenesis are decompensated and the cortical neurons are not protected against destruction. In this case, the low BDNF level is not sufficient to maintain efficiently the processes of neuro-trophicity and neuro-regeneration. Administration of the neurotrophic factor protects sensible cerebral tissues against hypoxic-ischemic injuries.

KEY WORDS

BDNF, NEUROTROPHIC FACTORS, PERINATAL HYPOXIC-ISCHEMIC ENCEPHALOPATHY, CENTRAL NERVOUS SYSTEM

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NOVELTY OF THE TOPIC

Neuropsychical pathology (npp) represents one of the most frequent causes of invalidation in children from Republic of Moldova.

Perinatal hypoxic-ischemic injury (PHIE) constitutes the most important cause of invalidity and infantile mortality because of its consequences on the central neurvous system (CNS) [4, 6, 13, 23].
They have estimated that 2 to 4 % of in term newborns (**n.b**) present hypoxia during birth or shortly before birth [23].

– After years of experimental research on animals with a series of pharmacological agents (free radical synthesis inhibitors, free radical sweepers, antagonists of glutamate-type amino acid excitators, blockers of calcium canals, inhibitors of nitric oxide), glucocorticoids, and Phenobarbital, they have shown the inefficiency of the latest in prevention and treatment of secondary cerebral injuries PHIE.
– Hereby, the most important problem of neonatology and neuropediatries remains unsolved [8, 21, 23, 26].

At the present time, a number of studies confirms the role of neurotrophic factors in the maintenance of neurotrophicity in hypoxic-ischemic injuries of the brain [3, 21, 26]. Though, until now, no estimative clinical studies regarding neurotrophic disorders correlated with PHIE have been done.
The experimental studies on neurotrophic factors represent the main part of all fundamental researches in neurobiology.
Experimental results on animals and cells' cultures have shown that these factors have spectacular effects with perspectives of therapeutic application in the near future [21].

► The present study is an important scientific value due to the actuality of the broached direction, therapeutic and prophylactic perspectives in the treatment of child neurologic pathology.
The physicians will be able to use the obtained results in order to see more clearly the injury mechanisms of PHIE.

STUDY OBJECTIVES

Assessment of BDNF level in the serum of children with diverse grade perinatal hypoxic-ischemic injuries in acute and recovery periods. The study will confirm the neuroprotective, neurotrophic, neuroregenerative, neurometabolic roles and the therapeutic and prophylactic perspectives of BDNF.

MATERIALS AND RESEARCH METHODS

182 children aged from 7 days to 12 months (study lot – st.lt), who suffered from diverse grade PHIE of the CNS were investigated.
The allotment in sub lots (sbl.) according to age and clinic diagnostic: **7 days – 1 month** (nr. 46) children with PHIE acute period (PHIE gr.I / sbl.I – nr. 16, PHIE gr.II / sbl.II – nr. 15, PHIE gr.III / sbl.III – nr. 15); **1 – 3 months** (nr. 46) with disorders of muscle tonus (DMT) and npp (gr. I / sbl.I - nr. 16, gr. II / sbl.II - nr. 15, gr. III / sbl.III - nr. 15); **3 – 6 months** (nr. 45) with DMT and npp (gr. I / sbl.I - nr. 15, gr. II / sbl.II - nr. 15, gr. III / sbl.III - nr. 15); **6 – 12 months** (nr. 45) with DMT and npp (gr. I / sbl.I - nr. 15, gr. II / sbl.II - nr. 15, gr. III / sbl.III - nr. 15).
Children aged 1-12 months (recovery period) presented DMT and npp as consequences of PHIE.
– 60 healthy children formed the control lot (control group – ctrl.gr.).

Children with intrauterine infections, metabolic and toxic encephalopathies, and congenital brain malformations were excluded from the study.

In conformity to the established goals, children from each lot passed the clinical, functional and laboratory investigations. The neurological statute was assessed at children aged 7 days – 12 months with a periodicity of 3 months. The anamnesis was collected in order to assess the dynamic of motor (mt.) and neuropsychical (nrps.) acquisitions.
We appreciated the following domains: cognition, expressive language, receptive language, fine motricity, gross motricity, etc.
An age-related scale of evaluation of the development degree of nrps and mt was elaborated – tests, appreciated with 1, 2, 3 and 4 points.

Performance acquisitions according to age – 4 points (pt.); I grade performance difficulties – 3 pt., II grade – 2 pt., III grade – 1 pt.
The goal of this evaluation was to highlight the alerting neurological signs, level of maturation of the CNS and the risks of development of neurological after-effects in children that had suffered from PHIE.

Transfontanelar NSG, EEG and brain TC were done in the dynamics of the first year of life.

The plasma and BDNF level from the serum of the two lots of children were studied. Laboratory examinations were effectuated on the immunological analyzer STAT FAX-303 by the immunoenzymatic analysis method (ELISA).

Statistical procedures were effectuated on the personal computer IBM PC using the software STATISTICA 6.0.
The t Student criterion was used to appreciate the level of differentiations between the average values.

RESULTS

Documentation of antecedents was prepared in order to obtain a more correct diagnose.
87% from st.lt. were children with an unfavourable obstetrical anamnesis, 28% of whom suffered during birth.
The most frequent maternal affections during pregnancy were: gestational toxaemia (59%), anaemia (62%), and arterial hypertension (38%).
We have noted that the most frequent pathology of the labour and expulsion, like: insufficiency of contraction forces, procidence of cordon, difficult extraction were met in **37% of cases**.
We noted peri- and intrapartum precedents in only 2% of ctrl.gr. ($p < 0,01$).
Likewise, fetal pathology may be a provocative factor of hypoxic-ischemic injury suffering.

PHIE symptoms in acute period at children from st.lt

Symptoms	PHIE (lst.lt– 46 children)					
	sbl. I, nr.16	%	sbl. II, nr.15	%	sbl. III, nr.15	%
Neuroreflector hyperexcitability	+	98	-	-	-	-
Transitory reflexes	present	96	dejected	97	inhibited	100
ROT	exaggerated	96	dejected	94	inhibited	100
Hypotony	soft	87	expressed	100	diffuse	100
Sympathetic hyperactivity	+	89	-	-	-	-
Lethargy/stupor	-	-	+	73/37	+	31
Convulsions	-	-	±	34	+	58
Coma	-	-	-	-	+	69
Neurovegetative disorders	-	-	averagely expressed	43	expressed	100
Signs of affectation of cerebral trunk	-	-	softly and averagely expressed	45	expressed	92
Duration on clinical manifestations	under 2-4 hours	86	under 1 week	79	over 1 week	96

TAB. 1

We noted at children from st.lt.: premature birth (23%), cord congenital diseases (4%), haemolytic anaemia (8%), hyperbilirubinemia (12%).
The low Apgar score allowed us to determine n.b's statute and a suspicion of a fetal sufferance in the st.lt (67%) in comparison to ctrl.gr. (4%).
Neurological symptoms had lasted during the 2-4 weeks of life at children from st.lt. (TABLE 1).

Clinical manifestation at children with PHIE had ameliorated by the age of 2 weeks-1 month, according to the degree of the PHIE. Cerebral functions had remained severely affected in the difficult forms of PHIE.
Children from sbl. II and III were presenting severe neurological syndromes during the retrieval period.

In order to screen the nrps. and mt. development the reflexes (rf) of CNS morphofunctional development were assessed: rf of extension of superior limbs, heel rf, rf of vertical maintenance, supra pubic extensor rf, crossed extension rf, Galant rf, Moro rf, cervical tonic rf, hand grasping rf, plantar grasping reflex.
Reflexes of development have an important clinical signification in the assessment of CNS maturation, presenting very often the locating value of injuries. Positive rf at corresponding age gained 4 pts.; rf. present at the time when it should disappear – 3 pts.; present 3 months after the time when it should disappear – 2 pts.; present after 6 months – 1 pt.

The pathologic response of 3 primitive reflexes represents valuable information to forecast the nrps and mt deficits.
Motor acquisitions were evaluated: maintenance of the head, rotation from belly to back, rotation from back to belly, sitting with support, crawling, pace. – Normal motor performances' development was quantified with 4 pts.; 2 months delay – 3 pts.; 4 months – 2 pts.; 6 months and more – 1 pt.
Non acquisition of motor performances at the physiological age suggested the idea of a severe neurological sufferance and, respectively, the retention of CSN maturational process. Children with PHIE from gr.II presented in 4% of cases, and those from gr.III in 38%: MTD, anomalies of developmental reflexes, non acquisition of nrps and mt performances at physiological age.
We observed that nrps and mt developmental indexes of sbl.I were diminished insignificantly by the end of retrieval period in comparison to ctrl.gr. ($p<0,01$).
The most diminished indexes were appreciated in sbl. PHIE-II ($p<0,05$) and –III ($p<0,1$), in comparison to ctrl.gr.

Present day studies confirm the neuroprotective role of neurotrophic factors on CNS and peripheral.
In the present study we assessed BDNF levels in the serum of children with diverse grade PHIE. The obtained results are presented in TABLE 2.

BDNF level was assessed in st.lt and ctrl.gr. A statistically important variation of BDNF was established in the ctrl.gr.: 1001,8 pg/ml (at n.b.) and 1011,9 pg/ml (between 6 and 12 months), minimal value – 989 pg/ml (at n.b.), maximal value – 1021,7 pg/ml (between 6-12 months).
The threshold of this sub lot had stable values increasing only with 0.99%.

Thus, it was confirmed that in the period of growth of the healthy child BDNF is the neurotrophin responsible for a normal nervous system development (see TABLE 2).

It was established that in sbl.I the average BDNF level was smaller in comparison to ctrl.gr and it varied between 945,6 pg/ml (at n.b.) and 973,4 pg/ml (between 6-12 months), minimal level was 919,2 pg/ml (n.b.), and the maximal 991.2 pg/ml (between 6-12 months).
The threshold of this sub lot constitutes $p<0,05$, $p<0,1$.
The BDNF level in sbl.I (6-12 months) increased with 2,7% by the age of 1 year old in comparison of sbl.I (n.b.) and was 38,5 pg/ml (3,8%) more diminished in comparison to ctrl.gr.
The statistically significant variation between the minimal and maximal level at newborns constituted +26,4 pg/ml, and at the age of 6-12 months +18,7 pg/ml.
The BDNF values at children from sbl.I approached indexes of healthy children.
BDNF level from the serum of sbl.I was 3,8% smaller in comparison to ctrl.gr. (see TABLE 2).

Neuro-psychical and motor developmental levels in sbl.I were assessed in conformity to the scale: with 3 (6% of cases) and 4 (94%) points.
Neuro-psychical abilities were mildly affected at 4% of children.

BDNF level in children under 12 months old increased approaching indexes on

BDNF level in serum of children with diverse grade PHIE, at different ages, M+m

Child's age	Form of the disease			
	Control lot nr. 12	Study lot- sbl.I, nr. 14	Study lot - sbl.II, nr. 13	Study lot –sbl.III, nr. 14
	Serum level of BDNF, pg/ml			
7 days – 1 month	1001,8±12,8*	945,6±26,4***	863,1±41,8***	724,7±63,5***
1 – 3 months	1005,9±9,8*	972,5±17,2**	882,9±48,7***	764,5±46,6***
3 – 6 months	1007,2±7,3*	972,5±18,4**	886,5±51,6***	785,4±49,9***
6 – 12 months	1011,9±9,8*	973,4±18,7**	879,8±52,8***	788,9±54,7***

*- $p<0,01$ **- $p<0,05$ ***- $p<0,1$

TAB. 2

children from ctrl.gr.
There was observed a quick amelioration of EEG and imagistic interpretations. Neurological disorders would be functional in children with insignificantly diminished BDNF levels.
An important statistical variation between the average levels of BDNF ($p<0,01$) in children from sbl.I and ctrl.gr was observed.
By the age of 1 year the neurological after effects were minor in sbl.I.
Neurologic syndrome of these children would ameliorate rapidly after the administration of neuro-protectors at precocious stages.

► **Thus, it was confirmed that BDNF protects cortical neurons from destruction and ameliorates the neuroplasticity.**

We assessed that in sbl.II the average level of BDNF was lower in comparison to ctrl.gr. and it varied between 863,1 pg/ml (n.b.) and 879,9 pg/ml (between 6-12 months), minimal level was 821,3 pg/ml, and the maximum – 932, 6 pg/ml (6-12 months).
The threshold of BDNF in sbl.II constituted $p<0,1$.
In sbl.II, BDNF increases with 1,89% by the age of 1 year old in comparison to sbl.II (n.b.), being with 132,1 pg/ml (13.05%) lower than the ctrl.gr. level.

Statistically significant variation between the lowest and the highest levels at newborns constituted **+ 41,8 pg/ml**, and at 6-12 months old **+ 52,8 pg/ml**.

BDNF had maximal values in 72,2% of children from sbl.II by the age of 12 months, in 24,8% - average levels, and 4% - minimal levels (see TABLE 2).
Thus, in 24,8% of children (sbl.II) appeared a risk of retention of nrps and mt development, in 4% of cases it was important.
We appreciated an average and severe retention of nrps and mt development at children from this lot.
The level of nrps and mt development of children from sbl.II was evaluated in conformity to the scale: 4 (54%), 3 (42%), and 2 (4%) pts.
The level of influence of nrps and mt abilities in sbl.II was: mild (27%), average (15%) and severe (4%).
Following the comparative analyse, a statistically significant variation between the average BDNF levels was established ($p<0,05$) at children from sbl.II, that presented afterwards neurologic disorders, and ctrl.gr.
Neuropsychical and motor disorders were minor at children form sbl.II with higher levels of BDNF in serum.
Likewise, we observed at these children a rapid remission of NSG and EEG anomalies that indicates a favorable evolution of neurodevelopment.
Severe and average grade neurologic syndromes developed at children with minimal levels of BDNF, by the age of 1 year old. NSG and EEG anomalies persisted in evolution.

Thus, it was established that BDNF level will have a higher positive trend by the age of 12 months in children from sbl.I and II in comparison to ctrl.gr.
This explains that BDNF has neuroprotective function, through acceleration of myelinisation, reorganization and set-up of cerebral tissue neurons in injuries induced by hypoxi-ischemia.
Or, the mechanisms of neuroregeneration are more developed at children with neurological problems.

Average level of BDNF was lower in sbl.III in comparison to ctrl.gr, variations were between 724,7 pg/ml (at n.b.) and 788,9 pg/ml (between 6-12 months); minimal level 661,2 pg/ml (at n.b.) and maximum of 743,6 pg/ml (between 6-12 months). Threshold in sbl.III constituted $p<0,1$ (see TABLE 2).
BDNF had a 8,1% growth by the age of 1 in this sub lot in comparison to n.b, being 223 pg/ml (22,03%) lower than the assessed level in ctrl.gr.
Statistical variation between the minimal and the maximal levels constituted + 63,5 pg/ml in n.b, and + 54.7pg/ml at 6-12 month old children.
By the age of 12 months the BDNF level presented maximal values at 40% of children from sbl.III. 60% of children of this sub lot had a high risk of development of nrps and mt retard.

Nrps and mt disabilities were diagnosed in 78%, 48% of whom were severe.
The level of nrps and mt development in this sub lot was appreciated in conformity to the scale: 3 (24%), 2 (52%) and 1 (24%) points.
The largest part of children from this sub lot presented important neurologic after effects by the age of 12 months old.
Very low levels of BDNF were observed at children with severe mt retard and epileptic seizures.
At the age of n.b., BDNF (lower than 790 pg/ml) could be a **marker of development** of nrps and mt retard in children. We established an important statistical variation between the average levels of BDNF of children from sbl.III, with severe neurologic after effects, and those from ctrl.gr ($p<0.1$).
Nrps and mt disabilities were severe in children with minimal levels of BDNF.

Previously, it was confirmed that BDNF protects cerebral tissue from destruction. It is said in one of studies: "*One of the premises regarding the mechanism of neuroregeneration evoked by an injury consists in the fact that injured cells induce the liberation of neurotrophic factors to stimulate the neurogenesis. Extracellular purinic nucleotides exercise multiple neurotrophic actions in CNS being mediated through activation of purinergic receptors and mediate the liberation of neurotrophic factors to encourage the regeneration of injured olfactory epithelium*" [20].
– But, probably, neurotrophic processes decompensate and cortical neurons are not protected any more when BDNF levels are very low.
In this case, low levels of neurotrophic factors are not sufficient to maintain the neurotrophicity and the neuroregeneration. The brain adaptability is diminished. In this case, there is a probability that BDNF concentrates maximally in injured cortical areas, where structural and functional reorganization of white and grey substances takes place.
Thus, at children with hypoxi-ischemia, destructive processes prevail on those

of neuroregeneration. It is obvious that modifications of cerebral tissue in PHIE gr.I and II (partial) are functional and short termed.
In PHIE gr.II (4%) and III the modifications are structural and long termed.

Low levels of BDNF were frequently appreciated (authentic statistical data) at children with severe forms of PHIE that are often associated with disorders of conscience, convulsive manifestations, disturbances of muscular and reflex tonus. Likewise, low levels of BDNF were identified in n.b. presenting pathologic EEG patterns (a marked decrease of wave amplitude and frequency) and with neuro-imagistic data attributed to cerebral hypoxi-ischemia (increase of ecogenity of cerebral tissue, edema, stasis, ischemia in neural tissue).
Low values of BDNF confirm the presence of severe injuries of cerebral tissue by the age of 12 months.
It is known that BDNF manages processes of neurogenesis, when BDNF decreases the latest decompensate.
The present study confirms that BDNF is an important marker of destructive lesional sufferance of cerebral tissue.
Thus, BDNF level would be different according to the degree of affectation of cerebral tissue in hypoxic-ischemic injuries. This one increases significantly, but in different proportion, by the end of the first year of life.
BDNF level in children from st.It remains decreased in comparison to those from ctrl.gr. Very low levels are preserved at children with severe mt. deficit and at those with recurring epileptic seizures.
It is possible that the retention of synthesis of neurotrophic factors could be one of the most important causes that stops the nervous cell maturation processes at children with PHIE.
Likewise, **low BDNF level** (lower than 790 pg/ml) **has a predictive value** for the nrps and mt retard at children with severe PHIE. Decreased BDNF level associated with an aspect of anomalies and diminished voltage of EEG waves is an unfavorable prognostic indicator.

We made the comparative analyze of low BDNF levels in sbl.I, II and III.
It shows that the probability of decreased BDNF level constitutes 23,34% at children from sbl.II and 85,4% at those from sbl.III in comparison to sbl.I (3,42%). The probability of decreased BDNF level is high in sbl.III, especially at children with severe neurologic disorders.
Synthesis and secretion of BDNF decreases seriously at children with severe hypoxic-ischemic injuries. Because of low BDNF level neuroprotective and neuroregenerative processes are inhibited. A series of anomalies of neurotrophic factors may accompany the hypoxic-ischemic aggression.
This contributes to stoppage of nervous fiber maturation and to development of severe neurologic after effects. The probability of development of neurologic after effects in PHIE-II is lower. Probably, the processes of neuroprotection in these children are compensated by the acceleration of neurotrophic factors' synthesis. In this case, BDNF is maximally concentrated in affected cerebral areas where it participates intensely to the processes of neuroregeneration and re-modulation.
It is confirmed, in multiple studies, that 2 hours after ischemia an increase of BDNF imunointeractive cells is observed in *singular gyrus* and *frontal cortex except* for injured area [21].
It is possible that there exists an inferior limit of BDNF level and under this limit the processes of neuroregeneration and neuroregulation are irreversible. Or, high BDNF level protects the brain from destruction, sustains the survival and differentiation of neurons, increases neural cell's resistance to injuries induced by ischemia.
Thus, we could establish a positive correlation between neurologic after effects of PHIE and concentration of BDNF in blood.
Therefore, it is the case to administrate exogenous neurotrophic factors before the appearance of cerebral lesion changes, that is to say in the first minutes or hours following the apparition of cerebral hypoxic-ischemic injuries.

DISCUSSION

Aetiological factors, which may conduct to affectation of nervous system of fetus and n.b, may influence during intrauterine, intranatal and neonatal periods [23, 31, 32].
Lesion structure of the brain of n.b. changes considerably in dependence on gestational age. The consequences of perinatal pathology determine the base neurological disorders of very young children, thus occupying the first place among cerebral pathologies [24].

Neonatal encephalopathy is a heterogeneous syndrome characterized by dysfunctional symptoms of CNS in term or under term n.b. (> 36 weeks of gestation). Children with neonatal encephalopathy may be exposed to abnormalities of conscience level, convulsions, reflex abnormalities, apnea, and alimentation difficulties [23]. Neonate encephalopathy may be the result of a variety of conditions and often remains inexplicable. Responsible of these are asphyxia and PHIE. Cerebral injury causes neurological deterioration in n.b., who cannot be understood often. Neonate encephalopathy's pathogenesis is discussed everywhere [20, 23, 31]. Neonate encephalopathy causes neurological deterioration in n.b., that leads very often to cerebral palsy (CP), a heavy burden for family and society [3, 4, 23].

Encephalopathic n.b. may have an abnormal state of conscience (ex: hyperalerted, irritable, lethargic, obtuse), with respiratory and alimentation difficulties, reduced tonus or convulsive activity. Often, these n.b. present a low Apgar score and a weak or absent cry [23].
An important part of these symptoms were observed at children from study sublots.

The lesion of CNS of the child would have as consequences the nrps and mt retard [3, 4, 31].
Injuries in immature period have a different pattern from those in adults (while immature, the affected zone

would be symptomatically nonfunctional), especially in children with neurologic pathologies [23].
Thus, it is very important to know the main reflexes regarding nrps development of the child at different ages.
A scale of evaluation of nrps and mt developmental level of children was elaborated in study lot.

The diagnose of neonatal encephalopathy imposes the research of potential aetiologies. An evident and histological examination of placenta and cordon could deliver evidences of possible causes like vascular or infectious lesion of placenta, or thromboses of cordon [23].
A detailed maternal and familial history is recommended, including history of thromboembolic disorders, anterior losses of pregnancy, maternal infections or drug abuse.
Sampling is done to determine arterial pH and base deficit of cordon. The presence of oliguria, cardiomiopathy, abnormal function of the lung may suggest a global hypoxico-ischemic event [23, 31].

Neuroimagery has become more important in the evaluation of neonatal encephalopathy and can deliver information concerning the type and the synchronization of cerebral injuries [4, 23].
For example, several patterns of cerebral injuries were observed at in term and premature n.b., who are considered to be with typical hypoxic-ischemic injuries, bat not in all the cases with hypoxic-ischemic affection.
TC is responsible for doubtless diagnosis in precocious terms [23].
The brain of small child is an *object* of study. CNS affection would mark the retard in nrps development. The most favorable condition in neurological pathology is to speed up the maturation of nervous fibers at children with neurologic pathologies, in order to prevent CP, whose deficiencies are evident by the complete maturation of CNS structures. The appreciation of peculiarities of neuroontogenesis in children that suffered from perinatal hypoxic-ischemic cerebral lesion is possible due to nowadays achievements in neurochemistry, related to researches of neurotrophic factors (BDNF, FCNT, etc.) [8, 14, 17, 18, 21, 26, 28, 29].

The consequences of cerebral perinatal affection, in most of cases, would be determined by a precocious diagnosis of cerebral injuries and the efficiency of the treatment in the period of intensive maturation and development [23, 31].

Neurotrophic factors of the brain could be very important in pathogenesis of perinatal hypoxic-ischemic injuries.
Experimental studies have shown that these factors play an important role during the ontogenetic development, in survival maintenance and the differentiation of neurons [1, 7, 8, 9, 10, 16, 21]. Especially important are the results of researches on animals that proved that neurotrophic factors have therapeutic neuroprotective effects in numerous pathologic conditions of the brain [21].

BDNF initially was described as neurotrophic factor with effects of promotion of survival of sensitive neurons from spinal ganglions. Later it was observed that BDNF influences on every classes of sensitive neurons, promotes differentiation of motoneurons *in vitro* and protects these cells from apoptosis and from death induced by axotomy in n.b animals [21].

BDNF is the most prevailing neurotrophin in the brain.

BDNF's RNA messenger was identified in various structures of the rat brain [3].
Thus, the treatment with kainat (an excitatory aminoacide, aromatic analog on glutamic acide) determined an intense presence of BDNF in pyramidal cells of different cerebral structures, a strong argument in favor of neurotrophic role of BDNF in maintaining neurons and synapses in adult animals [3, 21].
In several studies they investigated the role of BDNF in maintaining the survival and the differentiation of cholinergic neurons of basal and anterior region of anterior brain [7, 21, 22].

Several groups of studies proved that cerebral intra-ventricular infusion with BDNF at rats and monkeys can prevent the atrophy and the loss of phenotypic markers of septal cholinergic neurons, resulting after axotomy [21].

Multiple studies demonstrated the neuroprotective role of BDNF in cerebral ischemia. Thus, Arai *et* Al. [1] confirmed that the intensification of BDNF's presence and his receptors is produced in areas beyond infarct.
Another study notes that one of the premises concerning neuroregenerative mechanism launched by the lesion consists in the fact that injured cells liberate neurotrophic factors to stimulate neurogenesis [21].
A more recent study argues that assessed BDNF's levels are correlated, at least partially, with the resistance of cells face to injuries induced by ischemia, and are in correspondence with the existence of neurotrophic role of BDNF [5].
Likewise, the same author confirms that 2 hours after ischemia they observed an important increase of the number of immunoreactive cells to BDNF in *cingular gyrus* and *frontal cortex* beyond injured area. In animals with cortical lesions they observed a disappearance of immunoreactive cells to BDNF in *striatum* in 2-24h, while animals with injured *striatum* didn't present that modification.
After 2-24h they observed strongly immunoreactive fibers along myelinated fascicles situated medially to *striatum*, in anterior commeasure and in *corpus callosum* ipsilateral to OACM. BDNF levels went to 133-213% after 2h in *cingular gyrus* and *frontal cortex*, and diminished to 40% after 24h in *striatum*.
Thus, they noted the augmentation on BDNF protein 2h after ischemia.
The diminution of BDNF after 24h suggests a pronounced liberation or anterograde axonal transport in postischemic phase. BDNF modification after focal ischemia has a role in survival of cortical and *striatum* neurons [5].

BDNF infusion had been effectuated beginning with not long after the occlusion of middle cerebral artery (in rats) and had been continued for 24h, and after they assessed infarct's volume, in comparison to witness group to which they infused only vehicle.
A 33% diminution of infarct was noted [33].

Other studies on animals noted that BDNF has an effect of contraction on neural lesions determined by asphyxia [17].
In hypoxic-ischemic lesion, BDNF has different effects on the brain in development (n.b.), in comparison to the adult one. A single intraventricular injection of BDNF determines a vigorous phosphorilation of Trk receptors (responsible for BDNF) in multiple regions of rats brain starting from the 7th postnatal day.
Thus, BDNF protects the rat's brain from hypoxic-ischemic lesions from the 7th postnatal day. BDNF protects 90% of nervous tissue from loss of neurons caused by ischemic hypoxia, if it's administrated before hypoxi-ischimia, and 50% if it's administrated after infarct.

BDNF represents a potential treatment of asphyxia and other acute perinatal lesions [8]**.**

Likewise, recent studies confirm that transitory ischemia intensified the expression of BDNF gene both in *hippocampus* and *cerebral cortex*. That was mediated through receptors of glutamate of NMDA and non-NMDA type [30]. Another study shows that the prevention of neural death with BDNF after cerebral ischemia is associated with the increase of expression of its specific receptor Trk-B [9].
BDNF is widely spread in CNS and presents *in vitro* trophic effects on diverse type of cells including cortical neurons, *hippocampal* neurons, *cerebellum* neurons, etc.
In vivo, BDNF rescues motoneurons, *hippocampal* neurons and dopaminergic cells of *substantia nigra* from traumatic and toxic lesions.
The intraventricular pre treatment with BDNF reduced the dimension of infarct after focal cerebral ischemia.
BDNF has a neuroprotective role in ischemic cerebral vascular accidents [27].

In our study we noted low levels of BDNF in serum of children that suffered from perinatal hypoxic-ischemic lesions of II and III grades.
– This datum has a huge value for the prognosis of delayed neurologic after effects.

It is written in one study:
"*The finding of an effective treatment of chronic neurodegenerative diseases still represents an unreachable goal, mostly because of the multiple variables specific to these diseases. Recently, the attention focused on the role of neurotrophic factors in etiology of these diseases because of their role of survival of different cellular phenotypes under diverse adverse conditions including neurodegeneration. This article summarizes the current statute and the efforts of treatment of neurodegenerative diseases through the administration of exogenous neurotrophic factors as a tentative to resupply the trophic store, whose insufficiency may contribute to the development of disease. Although some promising results were observed in animal models, this alternative still faces some problems of discordance, more often, when it comes up to clinical application, probably because of unique nature of human being*" [10].

The development of nervous system is accompanied by a complicated consecutive process of commutation of sensibility to neurotrophins in certain populations of neurons.
It is possible that this complicated system calls for being regulated not only at the level of signal induction (i.e. at the level of neurotrophin's production), but also at the level of perception of this signal by neurons (i.e. at the level of production of receptors for neurotrophins). An insufficient supply of trophic factors has a role in the development of cerebral ischemic processes.
The level of neurotrophicity determines the alternative selection between genetic programs of apoptosis and anti-apoptosic protection, that influence on necrotic and reparative mechanisms.
The synthesis of trophic factors and their receptors in first minutes following the ischemia represents the natural protective reaction of the brain. Cerebral ischemia may not produce infarct changes for a long time because of rapid and active expression of genes through coding neurotrophins.
In case of occurrence of ischemic lesions the high level of trophic factors would assure the regression of neuropsychomotor disorders up to the conservation of morphologic defect that provoked it.

Several experimental studies argue that the administration of an excess of neurotrophic factors protect sensible cerebral tissues. The treatment of animals with neurotrophic factors before and 90 minutes following the transitory ischemia reduced effectively the volume and infarct area without affecting regional cerebral blood flow in comparison to control group or the group with untreated animals.
Thus, it appears that neurotrophic factors exert a strong neuroprotective effect from ischemic cerebral lesions [2, 10, 12, 25].

An alternative explication is that beneficial BDNF effects are due to amelioration of exitotoxicity [21, 31].
The antagonists of glutamate receptors reduce the volume of infarct [19, 21, 25].

BDNF may exert his neuroprotective effect in ischemia through other mechanisms, like: toxicity of nitric oxide. There exist solid proofs that the inhibition of enzyme responsible for synthesis of neural nitric oxide reduces the volume of infarct [21]. Thus, BDNF inhibits this enzyme and maintains the survival of neurons after avulsion of ventral root [22]. There also exists the possibility that BDNF increases the local blood flow. Although, the hemody-

namic effects of BDNF have not been investigated [21]. **Nevertheless, it's obvious that BDNF has marked therapeutic effects in cerebral infarct** [11].
There were studies on rats concerning the efficiency of growth factors in recuperation of neurologic deficiencies after cerebral infarcts. In this case, growth factors were administrated several days after the occurrence of ischemia.
In these studies, they didn't have the intention to reduce the infarct volume but to intensify neurologic retrieval.
In rats, there exist certain proofs that focal cerebral infarction is followed by neuronal budding and formation of new synapses in neighbourhood of the infarct, in the same hemisphere and in homolog regions from collateral hemisphere [11, 16, 33].

This budding and formation of new synapses may represent a neurologic retrieval mechanism after focal infarction [21].

These observations have led to the hypothesis that the administration of exogenous growth factors, that intensify the budding of axons and dendrites, could intensify the functional retrieval after cerebral ischemia [21].

Neuronal plasticity represents an adaptability of nervous system to diverse lesions trough the structural and functional reorganization in white and grey matter [7, 15, 21].

The potential to facilitate the neurologic recovery through manipulation of biologic adaptability of brain and spinal cord has become an obvious fact in clinic practice.
The most favourable condition is: utilization of all means of reduction of handicapping conditions and rendering of optimal integration possibilities in society to people with disabilities [7].
In order to optimize the process of recovery it is important to apply techniques of stimulation of neuroplasticity.

Growth factors represent a citoprotective treatment to limit the expansion of infarct's volume if they are administered in the first hours following the accident, and facilitate the functional recovery if they are administered in the first month after the accident [21].

Number of studies on models with animals suffering from cerebral hypoxia and/or ischemia confirms the neuroprotective and neuromodulator role of Cerebrolysine in treatment of cerebral ischemia. Cerebrolysine has certain effect on different species of animals: diminishes the mortality induced by cerebral ischemia to 50% [27]; prevents the formation of citotoxic edema [27]; protects pyramidal cells from ischemia induced lesions [28]; prevents the formation of free radicals during reperfusion after cerebral ischemia [28]; diminishes essentially the concentration of lactate in brain hereby suggesting a protective effect during hypoxic-ischemic episodes [21]; assures the survival and promotes the differentiation of neurons in the same way as natural neurotrophic factors [21]; it has a dose-dependent effect preventing the death of neurons induced by high concentrations of glutamate [12]; the treatment with Cerebrolysine determines a statistically important amelioration at the Barolin Scale of Neurorehabilitation in the first 7 days. This finding is extremely important because of the introduction of "therapeutic window" in the treatment of cerebral hypoxi-ischemia [21].

Thus, administered neurotrophic remedies would contribute to recovery of nrps and mt acquisitions at children with neurologic problems.

Neurotrophins are the remedies which would be successful in this area.

CONCLUSIONS

BDNF has a protective role in cerebral lesions induces by hypoxi-ischemia.
It is a neurotrophin with important qualities of encouraging of growth, processes of myelinisation, reorganization and restructuration of cerebral tissue neurons. The mechanisms of neuroregeneration are more developed in children with neurologic problems. One of the premises concerning the neuroregenerative mechanism evoked by lesion consists in the fact that injured cells induce the liberation of neurotrophic factors to stimulate neurogenesis. We have studied the effects of BDNF on CNS neurons. We suggest that BDNF encourages axonal survival and regeneration, the maturation and maintenance of CNS neurons, when the latest are affected by hypoxi-ischemia. The high level of BDNF protects the brain from destruction, upholds the survival and differentiation of neurons. Different-grade motor and neuropsychical disabilities would correlate with low BDNF levels in serum, lower than 720 pg/ml, and that represents a marker of retard in nrps and mt development of children with hypoxic-ischemic lesions of the CNS. Neurotrophic processes decompensate and cortical neurons are not protected from destruction when BDNF levels are very low. In this case, the low level of neurotrophic factor is not sufficient to maintain the processes of neurotrophicity and neuroregeneration. Thus, the processes of destruction prevail on those of neuroregeneration in children with advanced grade of hypoxi-ischemia. The research of an efficient therapy curing perinatal hypoxic-ischemic lesion of the CNS still remains an unreachable goal, mostly because of the variety of PHIE clinical symptoms. The administration of exogenous neurotrophic factors before the apparition of modifications induced by cerebral lesions, during the first minutes or hours following the apparition of cerebral hypoxic-ischemic injury **could ameliorate the neurologic after effects and diminish the motor and neuropsychical handicap**.

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