UDC 616

CHANGES FABRIC PROTEO AND FIBRINOLYTIC ACTIVITY AND A LEVEL METABOLITES NITROGEN MONOXIDE AT RATS OF DIFFERENT AGE GROUPS ON A BACKGROUND OF DEVELOPMENT OF AN EXPERIMENTAL DIABETES

Tymofiychuk I.R.
Ph.D.assistant of professor,
Bukovina State Medical University, Department of Physiology,
c. Chernivtsi, Ukraine, Teatralna str., 2
Semenenko S.B.
Ph.D., assistant of professor,
Bukovina State Medical University, Department of Physiology,
c. Chernivtsi, Ukraine, Teatralna str., 2
Boreiko L.D.
Ph.D, assistant of professor,
Bukovina State Medical University, Department of Nursing and Higher Nursing Education,
c. Chernivtsi, Ukraine, Teatralna str., 2
Slobodian X.V.
Ph.D., assistant,
Bukovina State Medical University, Department of Physiology,
c. Chernivtsi, Ukraine, Teatralna str., 2
Maruschak A.V.
assistant,
Bukovina State Medical University, Department of Physiology,
c. Chernivtsi, Ukraine, Teatralna str., 2

The resume. In article the data about change of a level fybrino and proteolytic are submitted to activity on a background of development of a diabetes at rats - males of different age groups. And as the data about a role of monooxid nitrogen in pathogenesis of developments of degenerate changes of hippocampus of a brain of development of experimentala diabetes are received.
Key words. A diabetes, nitrogen monoxide, fibrinolysis, proteolysis.

Aging is an important factor responsible for modification of brain's neurochemical status. Oxidative stress, which is the reason for degeneration of brain's tissue, can initiate the modification of proteo and fibrinolytic activity which can cause changes of homeostasis on the level of cells, tissue and entire human organism [1,2]. That's why the significance of exploring these processes is obvious. Interaction of proteolysis and fibrinolysis systems defines pathogenesis of different changes, the degree of damage to cells and also adaptation and endurance of injured brain's tissue.

Tissue proteo and fibrinilytic activity plays an important role as far as mechanisms of selective sensibility of brain's structures to pathological factors are concerned. The impairments of balance in protease-antiprotease system are particularly dangerous to nervous system. The changes of proteolytic activity when growing older and especially when having a disease such as: diabetes, stroke can be the reason for Alzheimer's disease in senior patients [3,4].

The last investigations settled that one of the most essential mediators in human body was nitrogen monoxide (NO) which is considered to be tissue hormone, that supports active vasodilatation. Under normal conditions nitrogen monoxide constantly produces in human brain and causes certain physiological effects NO-synthesis and activity of proteolytic enzymes are connected which can be the basis of neurodegeneration processes [5,6].

Diabetes is usually considered as metabolic disease connected with impairments of all exchanges. Liver cells play a pivotal role here as they produce great excess of glucose, when p-cells of pancreas produce insulin. Damage to these cells leads to gradual changes of all metabolic and transport processes which take part in glucose's exchange in human organism. Hyperglycemia in patients who suffer from diabetes mellitus oxidative stress, additional vascular pathology and, as a result, hypoxia of neurons and neurodegenerative, give the opportunity to suppose that neurons of frontal cortex and hippocampus, which are the most sensitive to hypoxia are involved into pathological process [7].

When searching for preparation, which reduce consequences of oxidative stress it is desirable to find the ways which can positively influence the processes of fibrinolysis and proteolysis. The status of fibrinolysis and proteolysis in patients at different ages who have diabetes mellitus is not learnt enough.

Our aim was to carry out analysis which clarified proteo- and fibrinolytic activity of hippocampus tissue on nitrogen monoxide synthesis in rats of different age groups when developing experimental diabetes [8,9].

Materials and methods. The investigation were carried out on non lineal laboratory mall rats at the age of one months (young animals), five months (adult animal) and eighteen months (old animal). Protamine sulfate was used to awake diabetes mellitus. There was intermuscular injection 1 mg/kg two times a day for 14 day. The animals which had the level of glycemia not lower then 10 Mm/l, were included in experimental grays.

Tissue fibrinolytic activity was determined according to production of plasmin. The activity of non-enzymatic fibrinolysis was designated on accordance with the degree of solutions colouring in alkaline nuclium when adding ε-aminocaproic acid. The difference between this two indicators constitutes the intensity of enzymatic fibrinolisis [10,11].

Proteolytic activity in homogenates of brains structures was determined due to the intensity of colouring after the reaction with azoalbumin, azocasein and azocol.

Concentration of nitrates and nitrites way counted due to using the

Fig.1. *Influence of an experimental diabetes on parameters fabric fibrinolytic activity in hippocampus areas at rats of different age (M±m, n=8)*

Conditions of experiment	summary fibrinolytic activity			non-enzymatic fibrinolytic activity			enzymatic fibrinolytic activity		
	CA_1	CA_2	CA_3	CA_1	CA_2	CA_3	CA_1	CA_2	CA_3
Young animals	36,6±2,85	37,9±2,85	38,1±2,85	18,7±2,18	17,98±2,18	17,98±2,18	19,0±2,12	1,89±2,12	19,04±2,1
Adult animals	41,7±2,87	40,7±2,87	39,78±2,8	18,47±1,4	17,99±1,44	17,99±1,44	22,0±1,51	21,36±1,51	21,2±1,51
Old animals	33,4±2,87	37,4±2,87	36,9±2,87	17,2±1,44	16,89±1,44	16,89±1,44	19,0±1,51	18,980±1,5	18,99±1,5
Young animals with DM	54,3±4,41 P_1<0,005	55,3±4,41 P_1<0,005	54,9±4,41 P_1<0,005	28,9±1,94 P_1<0,005	29,01±1,94 P_1<0,005	29,01±1,94 P_1<0,005	26,1±2,47 P_1<0,005	25,98±2,47 P_1<0,005	25,91±2,4 P_1<0,005
Adult animals with DM	49,4±2,74 P_2<0,05	48,4±2,74 P_2<0,05	47,98±2,7 P_2<0,05	22,8±1,39 P_2<0,05	21,08±1,39 P_2<0,05	21,08±1,39 P_2<0,05	25,9±1,30 P_2<0,05	26,09±1,30 P_2<0,05	26,09±1,3 P_2<0,05
Old animals with DM	33,8±2,87	34,8±2,87 P_3<0,05	34,8±2,87	15,2±1,44	14,2±1,44 P_3<0,05	14,2±1,44 P_3<0,05	19,0±1,51	18,99±1,51	19,01±1

The note: reliability of parameters at rats concerning parameters at animals without a diabetes:
p_1 - молодих тварин; p_2 - дорослих тварин; p_3 - старих тварин. Statistically probable parameters at p<0,05

reagent of in plasma. As this reaction is peculiar only for nitrites, the nitrates were reduced to nitrites by cadmiums granules. The amount of nitrates/nitrites was expressed in mcmoles/l

Experimental interferences and euthanasia were carried out according to international principals of European convention on protection of vertebral animals. Statistic processing of the result was carried out due to application programmes "Statistica 6.0" and "SPSS 13". Parametric t-criterium of Student was used for assessment of differences of the average values.

The result of investigation. Excessive activation and depression of tissues proteolysis can be an important factor of pathogenesis in nervous tissue. As a consequence of anticoagulative systems awakening, there is an increase in coagulative potential of blood, durable hypercoagulation which inevitably leads to crash of anticoagulative mechanisms conditioned by disbalance of haemostasis system.

Proteolytic activity according to lysis of high molecular weight proteins tended to be reduced in hippocampus area CA_2 and was the lowest in old age group similar peculiarities of proteolytic activity status were the same in hippocampus areas according to lysis of low molecular weight proteins in the area CA_3.

Age-related differences between reduction of summary and enzymatic fibrinolytic activity were express in hippocampus areas CA_1 and CA_2 of old rats, in the area CA_3 cogent changes were not observed. See fig. 1 and 2.

Constitutive changes of proteolytic and fibrinolytic activity correlated with changes of nitrogen oxide's metabolites in plasma of rats at different ages. See fig.3

The rats of old age group had these indicators 2,7 times more in comparison with young rats and 1,3 times less than adult ones had. Diabetes mellitus arose cogent increase in nitrogen monoxides level in adult rats by 2 and was the reason for decrease of this indicator by 1,5 in old ones. It's supposed that nitrogen oxides synthesis occurs when one has acute hypoxia which is protective as prevent the adhesion of trombocytes to endothelium. The gene eNOS is expressed when there's an impact of acute hypoxia. These data is also worth interest because. Synthesis of NO lowers adhesion of leucocytes to endothelium at the expense of synthesis depression and selectines secretion. Under the conditions of prolonged hypoxia, NO-synthesis is lowered, although it depends upon the organ, species and age of the animals, oxygen tension, duration of hypoxia. Besides hypoxia, such metabolites as lactic and pyruvic acids influence the organ under ischemia which leads to conformation changes and intercellular proteins are exposed to damage including eNOS that is escorted by decrease in nitrogen oxide's secretion. As the enzyme eNOS is absent, the stimulation of NO-synthesis doesn't give desirable result.

Conclusion. Diabetes mellitus has become the reason for changes as far as proteolytic activity is concerned. There was an increase in indicators of fibrinolytic and proteolytic activity. The investigation has designated individual differences of proteolysis and fibrinolysis in various hippocampus areas of young, adult and old rats. This condition points out age-related dependence of activity reduction and the amount of enzymes that can (along with accompanying pathology) can be the reason for accelerated aging of brain and neurodegenaration in this structure of brain.

Fig.3. Contents NOx in fields of hippocampus a brain on a background of development of an experimental diabetes (M±m, n=8)

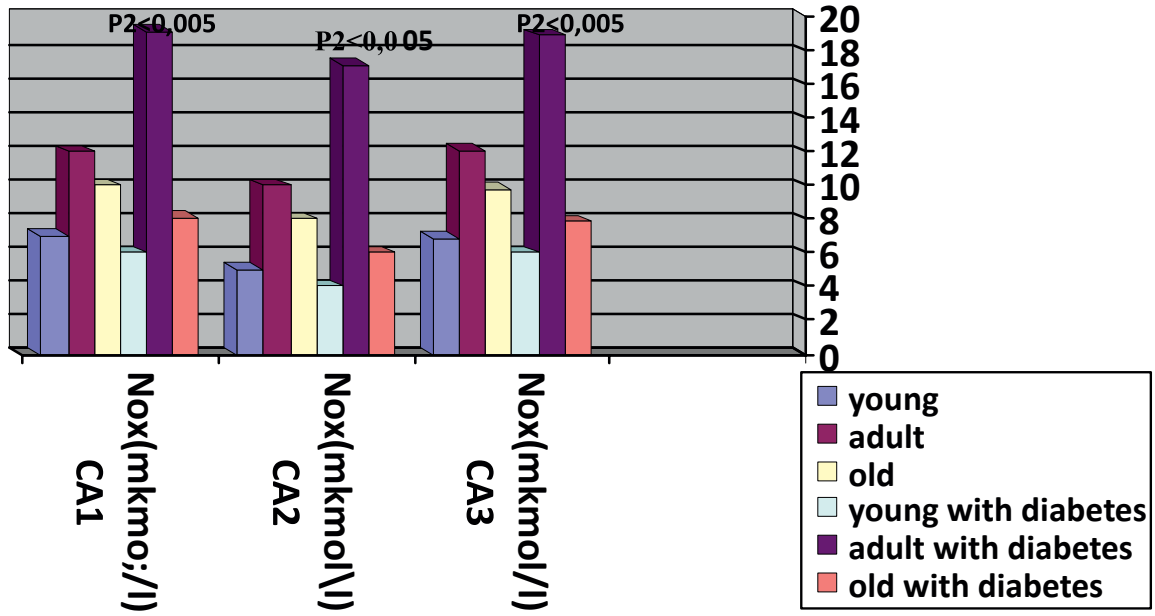

Fig.2. *Influence of an experimental diabetes on parameters fabric proteolytic activity in hippocampus areas at rats of different age (M±m, n=8)*

Conditions of experiment	Lysis of Low-molecular proteins, mkg azoalbumin / ml in one hour			Lysis of collagen, mkg azocol /ml in one hour			Lysis of High-molecular proteins, mkg azocasein / ml in one hour		
	CA_1	CA_2	CA_3	CA_1	CA_2	CA_3	CA_1	CA_2	CA_3
Young animals	2,03±0,0282	2,04±0,0172	1,59±0,028	0,274±0,005	0,25±0,005	0,274±0,005	2,21 ±0,102	1,09±0,092	2,41 ±0,102
Adult animals	1,9±0,0831	2,41±0,0831	1,45±0,034	0,228±0,009	0,21±0,009	0,228±0,009	2,27±0,12	1,89±0,0872	2,57±0,12
Old animals	1,34±0,0685	1,74±0,0685	0,78±0,0082	0,13±0,007	0,12±0,007	0,13±0,007	1,04±0,0988	0,89±0,002	0,94±0,0988
Young animals with DM	5,04±0,023 P_1<0,005	5,24±0,023 P_1<0,005	3,02±0,082 P_1<0,005	0,63±0,002 P_1<0,005	0,593±0,002 P_1<0,005	0,63±0,002 P_1<0,005	5,34±0,09 P_1<0,005	3,84±0,12 P_1<0,005	4,84±0,09 P_1<0,005
Adult animals with DM	1,2±0,012 P_2<0,05	1,9±0,012 P_2<0,05	0,98±0,0082	0,087±0,004 P_2<0,05	0,098±0,004 P_2<0,05	0,087±0,004 P_2<0,005	1,06±0,012 P_2<0,05	0,65±0,004 P_2<0,05	0,99±0,012 P_2<0,005
Old animals with DM	0,76±0,008 P_3<0,05	0,96±0,008 P_3<0,05	0,56±0,0065	0,07±0,002 P_3<0,05	0,069±0,002 P_3<0,005	0,07±0,002 P_3<0,05	0,65±0,003 P_3<0,05	0,34±0,005 P_3<0,05	0,7±0,003

The note: *reliability of parameters at rats concerning parameters at animals without a diabetes:*
p_1 - молодих тварин; p_2 - дорослих тварин; p_3 - старих тварин. Statistically probable parameters at p<0,05

LITERATURE:

1. *Role of nitric oxide – mediated glutathionylation in neuronal function. Potential regulation of energy utilization / Li-Peng Yap, J.V. Garcia, D.S. Han [et al.] // Biochem J. – 2010. – Vol.23. – P. 123–131.*
2. *Кургалюк Н.Н. Оксид азота как фактор адаптационной защиты при гипоксии / Н.Н. Кургалюк // Успехи физиологических наук. – 2002. – Т.33, № 4. – С.63–79.*
3. *Accumulation of calpain and caspase-3 proteolytic fragments of brain-derived αII-spectrin in cerebral spinal fluid after middle cerebral artery occlusion in rats / B.R. Pike, J. Flint, J.R. Dave [et al.] // Journal of cerebral blood flow&metabolism. – 2003. – Vol.24. – P. 98–106.*
4. *Protein aggregation and proteasome dysfunction after brain ischemia / P.Ge, Y.Luo, C.L. Lin [et al.] // Stroke. – 2007. – Vol.38, №12. – P. 3230–3236.*
5. *Tissue-type plasminogen activator in the ischemic brain: more than a thrombolytic / M. Yepes, B.D. Roussel, C. Ali [et al.] // Trends in neurosciences. – 2009. – Vol.39, №1. – P. 48–55.*
6. *Гоженко А.И. Коагуляционный гемостаз и окислы азота / А.И. Гоженко, С.Г. Котюжинская, В.П. Бабий // Клінічна та експериментальна патологія. – 2006. – Т.5, № 2. – С. 108–113.*
7. *Абакумов М.М. Оксид азота и свёртывающая система крови в клинике / М.М. Абакумов, П.П. Голиков // Вестник Российской АМН. – 2005. – № 10. – С 53–57.*
8. *Schawarz U.R. Taming platelets with cyclic nucleotides / U.R. Schawarz, U. Walter, M. Eigenthaler // Biochem. Pharmacol. – 2001. – Vol.2. – P. 15 – 28.*
9. *Species, strain and developmental variations in hippocampal neuronal and endothelial nitric oxide synthase clarity discrepancies in nitric oxide dependent synaptic plasticity / S. Blackshaw, M.J. Ellasson, A.Sawa [et al.] // Neuroscience. – 2003. – Vol.119, №4. – P. 979–990.*
10. *Structural features of ischemic damage in the hippocampus / A.G. Nikonenko, L. Radenovic, P.R. Andjus [et al.] // Anal Rec (Hoboken). – 2009. – Vol.292. – №12. – P. 1914–1921.*
11. *Куровська В.О. Вплив NO-залежних механізмів на показники фібринолітичної активності в гіпокампі щурів за умов ішемії-реперфузії головного мозку / Куровська В.О. // Бук. мед. вісник. – 2009. – Т.13, № 3. – С. 111–114.*

REFERENCES:

1. *Role of nitric oxide – mediated glutathionylation in neuronal function. Potential regulation of energy utilization / Li-Peng Yap, J.V. Garcia, D.S. Han [et al.] // Biochem J. – 2010. – Vol.23. – P. 123–131.*
2. *Kurgalyuk N.N. Oksid azota kak faktor adaptacionnoj zashchity pri gipoksii / N.N. Kurgalyuk // Uspekhi fiziologicheskih nauk. – 2002. – T.33, № 4. – S.63–79.*
3. *Accumulation of calpain and caspase-3 proteolytic fragments of brain-derived αII-spectrin in cerebral spinal fluid after middle cerebral artery occlusion in rats / B.R. Pike, J. Flint, J.R. Dave [et al.] // Journal of cerebral blood flow&metabolism. – 2003. – Vol.24. – P. 98–106.*
4. *Protein aggregation and proteasome dysfunction after brain ischemia / P.Ge, Y.Luo, C.L. Lin [et al.] // Stroke. – 2007. – Vol.38, №12. – P. 3230–3236.*
5. *Tissue-type plasminogen activator in the ischemic brain: more than a thrombolytic / M. Yepes, B.D. Roussel, C. Ali [et al.] // Trends in neurosciences. – 2009. – Vol.39, №1. – R. 48–55.*
6. *Gozhenko A.I. Koagulyacionnyj gemostaz i okisly azota / A.I. Gozhenko, S.G. Kotyuzhinskaya, V.P. Babij // Klinichna ta eksperimental'na patologiya. – 2006. – T.5, № 2. – S. 108–113.*
7. *Abakumov M.M. Oksid azota i svyortyvayushchaya sistema krovi v klinike / M.M. Abakumov, P.P. Golikov // Vestnik Rossijskoj AMN. – 2005. – № 10. – S 53–57.*
8. *Schawarz U.R. Taming platelets with cyclic nucleotides / U.R. Schawarz, U. Walter, M. Eigenthaler // Biochem. Pharmacol. – 2001. – Vol.2. – P. 15 – 28.*
9. *Species, strain and developmental variations in hippocampal neuronal and endothelial nitric oxide synthase clarity discrepancies in nitric oxide dependent synaptic plasticity / S. Blackshaw, M.J. Ellasson, A.Sawa [et al.] // Neuroscience. – 2003. – Vol.119, №4. – R. 979–990.*
10. *Structural features of ischemic damage in the hippocampus / A.G. Nikonenko, L. Radenovic, P.R. Andjus [et al.] // Anal Rec (Hoboken). – 2009. – Vol.292. – №12. – R. 1914–1921.*
11. *Kurovs'ka V.O. Vpliv NO-zalezhnih mekhanizmiv na pokazniki fibrinolitichnoї aktivnosti v gipokampi shchuriv za umov ishemiї-reperfuziї golovnogo mozku / Kurovs'ka V.O. // Buk. med. visnik. – 2009. – T.13, № 3. – S. 111–114.*