

Investigation of Pathogenetic Mechanisms of Experimental Cerebral Hemorrhagic Insult in Rats

Nana Tsanava, Vladimer Bakhutashvili

Department of Neurology, Tbilisi State Medical University, Georgia

Abstract

Have been studied oxidative metabolism and NO synthesis in rats during experimental hemorrhagic insult of the brain using the noninvasive photochemical and Electronic Paramagnetic Resonance (EPR) Methods. It is stated, that generators of oxygen active forms (ubisemiquinons, xanthinoxidase, Fe^{2+} and Mn^{2+} ions), excess amount of NO, and complexes of NO with heme- and nonheme iron are produced in cerebral cortex. Mitochondrial FeS containing proteins and cytochromes are nitrosylized; therefore mitochondrial electron transport and oxidative phosphorylation are suppressed. Presence of EPR signals of FeSNO and HbNO complexes pointing on nitrosilation of mitochondrial heme and nonheme iron containing proteins by free NO, make favorable conditions for apoptosis. In adjacent cells of the brain, that were not directly exposed to illumination, developed disorders are the same albeit of lesser intensity and delayed in development.

Keywords: *hemorrhagic insult, nitric oxide, oxygen reactive forms, EPR spectrum, brain, oxidative metabolism*

Introduction

Nowadays, the insult is one of the acute problems of modern life acquiring especial topicality in recent years.

It is known, that in the United States approximately 1 individual dies in every 3,5 minute with insult. About 55% of survivors after insult become invalid and reveal decreased capacity for work. Therefore, investigation of pathogenetic mechanisms of cerebral insult and the role of oxygen and NO reactive forms in particular is of great value.

According to above mentioned the purpose of this work was to investigate oxidative metabolism and NO synthesis in rats during experimental photochemical hemorrhagic insult.

Material and Methods

Experiments have been carried out in the Institute of Physiology of Georgian Academy of Sciences after I. Beritashvili.

Experimental and control groups of white rats with body weight 250- 300 g (total of 27 rats, 18 - experimental and 9 control) were investigated.

In present study, the new experimental model of brain's local insult was achieved using absolutely noninvasive photochemical method [1].

For realization of mentioned method, following procedures were carried out: After chlorhydrate narcosis (1ml of 4% solution per 100g body weight), during 2-3 minute at 37^o C, photosensitive dye - Bengale rose solution (0,13 ml of 0,75% solution per 100 g body weight) was administered in animals femoral vein. After, the rat was kept in stereotactic equipment followed by bearing of the cranium. Duration of exposure to illumination was 0,5, 1 and 1,5 hour.

From the halogen lamp source (243, 250 w), owing to optical ray conductive fiber (with diameter 2,5 mm), the final capacity for lighting (upon the cranium) was 0,64 w/cm².

Has been studied area of the brain's hemorrhagic insult directly exposed to illumination and adjacent area as well, in which pathological process extends with diminishing intensity.

Using the method, suggested by A. Vanin, content of NO in cerebral cortex was studied. According to the method, complexes of NO with Fe²⁺ and diethyldithiocarbamate were determined. They are iron and diethyldithiocarbamate mononitrosil complexes (EPR signal g=2,015).

With the purpose producing mentioned complexes, in experimental rats the intravenous injections of natrium diethyldithiocarbamate (20 mg/kg) was performed. Within 10 minute after injection animal was decapitated.

EPR specters of cerebral cortex tissue were obtained using the Radiospectrometer PЭ -1307 (Russia).

Results and Discussion

Received data clearly indicate, that after photochemical insult of the brain in the locus of illumination mitochondrial respiratory chain is disordered, electron transport at NAD.H ubisemiquinone-oxidoreductase locus is delayed leading to accumulation of ubisemiquinons with features of superoxidradical generators. At the end of the illumination it is added by decreased intensity of mitochondrial respiration (revealed by decreased EPR signals of free radicals and NAD.H dehydrogenase FeS centers).

All of these in turn should determine decreased synthesis of macroergic compounds, accumulation of hypoxantine and transformation of xsantinede-hydrogenase into xantinoxydase, revealed by Mo5+ containing complexes in the EPR spectrum of cerebral cortex.

In the late period of illumination (after 1 and 1,5 hour exposure) the quantity of less oxidized forms of metal ions with changeable valence (Fe²⁺, Mn²⁺) increases. These ions, as is known are powerful inductors of free radical oxidation (*Tab.1*).

After 0,5 hour exposure to illumination, production of NO increases excessively and correlates with duration of

exposure. Production of NO is much more exaggerated in case of prolonged illumination (after 1 hour exposure).

It is remarkable, that after 0,5 hour exposure, in the EPR spectrum of the brain, the nitrosil complexes of nonheme iron (FeSNO) are appeared reflecting nitrosilation of mitochondrial FeS containing proteins - NAD.H dehydrogenase and succinic dehydrogenase [2].

At this instance, electrons shift to central atom, localize and thereby delaying their transport among carriers of mitochondrial respiratory chain causing reduction in intensity of mitochondrial respiration. This last is revealed by decreased intensity of EPR signals of free radicals and NAD.H dehydrogenase FeS centers.

In the late period of observation (after 1 and 1,5 hour exposure), in EPR specter of radiated cerebral cortex, the nitrosil complexes of heme iron HbNO are detected, that is probably determined by interaction with mitochondrial heme containing cytochroms of NO.

It is well known, that the physiologic concentrations of NO reversibly inhibit cytochrom C oxidase (IV complex), which is probably the physiologic reaction directed towards decrease of oxygen consumption without change in ATP production. However, prolonged release of NO can lead to irreversible inhibition of I complex via the nitrosilation of critical thiols in enzymatic complexes, which has been shown in our experiment after 1-hour illumination.

Thus, excess amount of NO can produce early alteration of mitochondrial functions; decrease its energetic balance or intramembrane potential thereby stimulating apoptosis in brain cells. [3]

On the other hand, NO produces irreversible inhibitors of cytochrom oxidase (HbNO complexes), therefore NO is able to promote release of apoptotic factor (cytochrom c) from mitochondria that also leads to apoptosis of brain cells.

As far as the model of experimental photochemical insult has been used in an attempt to create the model of cerebral hemorrhagic model, that had been proved by widespread hemorrhages in illuminated area after section of experimental animals, it is expected that developed hematoma due to compression of surrounding tissue results in hemodynamic disorders and alteration of metabolism of adjacent tissue.

N	FREE RADICALS G=2,00	FES G=1,94	MN =2,14	MO G=1,97	FE G=2,25	NO G=2,01	FESNO G=2,03	HBN G=2,01
---	-------------------------	---------------	-------------	--------------	--------------	--------------	-----------------	---------------

Control	9	13,0±0,5	12,0±0,5	21,5±0,7	13,8±10,5	--	--	16,9±0,8	--	--
1										
<i>Illuminated area</i>										
0,5 hour	6	12,3±0,3	12,0±0,5	21,3±0,5	13,0±0,8	--	--	20,8±5,9	--	--
2		P _{1,2} >0,1	P _{1,2} >0,1	P _{1,2} >0,1	P _{1,2} >0,1			P _{1,2} <0,02		
1 hour	6	10,7±0,9	10,0±0,5	23,6±1,0	16,0±0,2	1,1±0,5	90,0±5,0	33,5±1,2	--	--
3		P _{1,3} >0,05	P _{1,3} <0,02	P _{1,3} <0,05	P _{1,3} <0,02			P _{1,3} <0,001		
1,5 hour	6	8,0±0,5	10,0±0,5	25,0±1,0	12,0±0,9	2,2±0,5	100±5,5	26,6±1,0	15,0±0,5	12,5±0,8
4		P _{1,4} <0,01	P _{1,4} <0,2	P _{1,4} <0,05	P _{1,2} <0,001			P _{1,4} <0,001		
<i>Adjacent area</i>										
0,5 hour	6	6,9±0,1	11,8±0,5	13,1±0,7	17,6±2,6	--	--	25,1±2,1	10,8±0,5	17,5±0,5
5		P _{1,5} <0,001	P _{1,5} >0,1	P _{1,5} <0,001	P _{1,5} >0,1			P _{1,5} <0,001		
1 hour	6	10,0±0,1	10,2±0,2	14,0±0,7	17,6±1,6	2,8±0,3	170,0±20,0	44,0±8,6	15,0±0,5	14,5±0,8
6		P _{1,6} <0,001	P _{1,6} >0,1 P _{5,6} <0,001	P _{1,6} <0,001	P _{1,6} >0,05			P _{1,6} <0,001		
1,5 hour	6	2,0±0,1	8,0±0,6	9,7±1,2	19,3±0,6	2,6±0,9	120,0±20,5	17,2±1,8	18,0±0,3	20,0±0,8
7		P _{1,7} <0,001	P _{1,7} <0,001	P _{1,7} <0,001	P _{1,7} <0,001			P _{1,7} >0,1		

Tab.1 Changes of Paramagnetic centres of the brain in rats during experimental hemorrhagic insult.

Above mentioned has been revealed in experiment by changes in metabolic paramagnetic centers of adjacent cells of illuminated area.

Noteworthy, that above mentioned disturbances develop more later than in area directly exposed to illumination and is of less intensity, however, as had been shown, after 1,5 hour exposure to illumination, disorders in mitochondrial respiratory chain at NAD.H ubiquinone oxidoreductase locus develop in adjacent areas of the brain as well revealed by decreased intensity of free radical EPR signals and half width (H) of it, and increased intensity of NAD.H dehydrogenase FeS centers EPR signals.

This last contributes to production of superoxidradical generators - ubisemiquinons, decreased production of macroergic compounds, increased containment of xanthinoxidase, intensification of free radical- or lipid peroxidation, inactivation of mitochondrial superoxididismutase and membrane structure destruction revealed by increased intensity of EPR signals of Mo⁵⁺ containing complexes, Mn²⁺ and Fe²⁺ ions correspondingly.

After 1 hour of observation, intensity of NO synthesis in adjacent cells of the brain that are not directly exposed to illumination, increases as well, which is detected by presence of EPR signals of

FeSNO and HbNO complexes pointing on nitrosilation of mitochondrial heme and nonheme iron containing proteins by free NO, thereby making favorable conditions for apoptosis in adjacent cells of the brain.

It is remarkable, that due to formation of HbNO and FeSNO complexes, containment of free NO in tissue decreases.

Conclusions

- After 0,5 hour exposure to illumination generators of oxygen active forms (ubisemiquinons, xanthinoxidase, Fe^{2+} and Mn^{2+} ions), excess amount of NO, and complexes of NO with heme- and nonheme iron are produced in cerebral cortex.
- After prolonged illumination, mitochondrial FeS containing proteins and cytochromes are nitrotilized, therefore mitochondrial electron transport and oxidative phosphorylation are suppressed.
- In adjacent cells of the brain area that were not directly exposed to illumination, developed disorders are the same albeit of lesser intensity and delayed in development.

References

1. Watson B.D., Dietrich W.D., Busto R.D., Watchel M.S., Ginsberg M.D. - Induction of reproducible brain infarction by photochemically initiated thrombosis. - Ann. Neurol., 1985, N17, p. 497.
2. Brown G.C. - Nitric oxide regulates mitochondrial respiration and cell functions by inhibiting cytochrom oxidase - FEBS Lett. 1995, 369, p. 136
3. Cleeter M.W., Cooper J.M., Daryl-Usmar V.M., Moncala S., Shapiro A.N. - Reversible inhibition of cytochrome C oxidase the terminal enzyme of the mitochondrial respiratory chain by nitric oxide, Implication for neurodegenerative disease - 1994 FEBS Lett. 345, p.354

Изучение некоторых патогенетических механизмов при экспериментальном церебральном геморрагическом инсульте у крыс

Нана Цанава, Владимир Бахуташивили

Кафедра нервных болезней Тбилисского государственного медицинского университета,
Грузия

РЕЗЮМЕ

Методами фотохимии и электронно-парамагнитного резонанса (ЭПР) изучены окислительный метаболизм и синтез NO в головном мозге у крыс при экспериментальном геморрагическом инсульте. Установлено, что продукция генераторов активных форм кислорода (убисемихинона, ксантинооксидазы, Fe^{2+} и Mn^{2+} ионов), NO и комплексов NO с гемовым и негемовым железом усиливается в коре головного мозга. Наличие ЭПР сигналов FeSNO и HbNO создает условия для развития апоптоза. В соседних тканях облученного мозга развиваются те же самые изменения, хотя с меньшей интенсивностью и медленнее.

Ключевые слова: *геморрагический инсульт, окись азота, реактивные формы кислорода, ЭПР спектр, головной мозг, окислительный метаболизм*