საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 6, №3, 2012
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 6, no. 3, 2012

Medical Sciences

Effect of Some Pyrrole Derivatives on the Brain Cortex during Hemorrhagic Shock

Grigol Sulaberidze*, Murman Ghvaladze*, Eka Chkonia*, Tamar Gegeshidze*, Teona Buachidze*, Mamuka Chkhaidze*

** Departments: Functional Morphology; Pathology and Cytopathology; Medical Pharmacology; Infectious Diseases; Psychiatry and Narcology; Tbilisi State Medical University*

(Presented by Academy Member Fridon Todua)

ABSTRACT. During hemorrhagic shock (HS), in which the destructive process develops, the brain cortex sharply reacts to hypoxia. The pyrrole derivative PV-88 is characterized by Ca^{2+} slow L channel blocking effect. Subsequently, it protects the brain cortex cells from overloading by Ca^{2+} ions. Possibly, it also affects T channels due to the cerebral-protective effect of this class of preparations.

The experiments were carried out on male cats. In total three series of experiments were conducted with 5 animals in each: one series in conditions of HS, the second series at the 80th minute of HS against the background of verapamil action, and the third series as a result of PV-88 effect.

Modeling of the hemorrhagic shock development was maintained up to 40 mm Hg the mean arterial pressure (MAP), gradually decreasing during 1 hour (HS60). The blood exfusion at 40 ml/kg was done against the background of artificial ventilation, which made 130-150 ml/min. The arterial blood pH was maintained at 7.35-7.45, and PO_2 - 90 mm Hg and more. The cat's body temperature stability varied between 37.8° C and 38.2° C. In the right femoral artery MAP was measured invasively, and from the left one the blood exfusion was done. Verapamil at the dose of 0.25 mg/kg and PV-88 at the dose of 3.0 mg/kg dissolved in normal saline, the volume of which did not exceed 1/20 of the exfused blood, were administered into the cat's right femoral vein. The animal's death was caused by putting it asleep at the 80th minute from the start of blood exfusion. We took the left frontal lobe (*Lobus frontalis*) of the brain cortex for examination by the electron microscope. Our research shows that PV-88, which is an acidic nitrate, 1-3(diethylamide propyl-3(n-fluorophenyl-3-oxopropen)pyrrol, is characterized by a more marked protecting ability to astrocytes and neurocytes, than preparation verapamil. *© 2012 Bull. Georg. Natl. Acad. Sci.*

Key words: *hemorrhagic shock, cerebral cortex, verapamil, PV-88.*

Hypoxia, developed during the hemorrhagic shock, significantly affects the cerebral cortex functioning. At a sharp decrease of the activity of cardiovascular system a significant increase of Ca^{2+} ion amount is observed in the cerebral cortex cells, causing destruction of cell membrane of the astrocytes and neurocytes. The basis of this process is the depletion of the cell energy stock and profound destruction of microcirculation, which ultimately ends in irreversible process development. Ca^{2+} L slow chan-

nel blockers are used for acute functional disorders of cerebral blood circulation and homodynamic disorders. In this aspect, nimodipin is characterized by a marked efficacy in the treatment of subarachnoidal hemorrhage [1-2]. Based on the position of the functional activity, the central nervous system (CNS) viability is provided by *feed-back* type cycles as a unified ensemble. Centripetal striving for a state of shock from the middle of the brain reticular formations is subjected to modulated control. The cerebral cortex functioning is reflected at this level, which dramatically affects afferent ways; this process is stimulated by positive *feed-back* startup. Rapid manifestation of morphological damage to the neurons, organized in this entire microsystem, and their incompatibility with life indicates that the central nervous system is a crucial organ in the moment of the death occurrence. The *ensemble* of shock-producing information runs around the cerebral cortex once again and causes sharp, fulminant damage of central encephalic formations; in this direction the Ca^{2+} slow channel blockers completely change the gravity of irreversible process development. Their ability can be used in polymorphous manner toward the damage of other genes. This group of preparations and in particular PV-88 may be used effectively for prevention of dementias. Studies, conducted on animals, demonstrated that L-type Ca^{2+} blockers weaken *â*-amyloid oligomer toxicity, tau protein destructive effect and improve the autophagy (the basic process of abnormal protein liquidation). Frequent, repeated craniocerebral trauma significantly contributes to the development of dementia, (e.g. boxers' dementia), which is explained by the extra- and subdural hemorrhage and anoxia. Supposedly PV-88 due to its mechanism of action will have an important role in studies and may be used in treatment of dementia, which becomes more topical for the increase in the average age of life [3-4].

Materials and Methods. The experiments were carried out on male cats weighing 2.5-3.5 kg., which were not given food for 24 hours (receiving unlimited amount of water). General anesthesia was done with nembutal 35-40 mg/kg. Through the catheter, inserted in the left femoral artery the blood flowed into a glass vessel (cylinder), MAP was decreased to 40 mm Hg during 1 hour. The anesthetized animal was connected to the oxygen–air artificial breathing apparatus "Лада", the air ventilation was 130-150 ml / min/kg in a way that the arterial blood pH was 7.35-7.45 and PO_2 - 90 mm/Hg and more. Gas levels were monitored on the analyzer "Corning" (Great Britain). During the experiment the cat's body temperature was measured rectally, which amounted to 37.8-38.2^{0}C. Temperature regime was maintained constant because its change significantly affects the homeostasis of the cerebral cortex [5]. The blood exfusion was done with the catheter, inserted in the right femoral artery and made 40 ml/kg, while MAP was measured in the left femoral artery. Heparin 2000 ED/kg was intravenously administered before blood exfusion; when MAP was stabilized to 40 mm/Hg, the experimental animal was disconnected from the apparatus and verapamil administered intravenously at the dose of 0.25 mg/kg, and PV-88 3.0 mg/kg, dissolved in normal saline, the amount of which did not exceed 1/20 of the blood exfusion.

Three series of experiments with 5 animals in each series (n=5)were conducted: 1. Control when frontal lobe of the brain cortex at HS60 was examined; 2. The series of examination against the background of a comparably standard preparation verapamil after 20 minutes of its intravenous administration; 3. In the same conditions the examination was done against the background of PV-88 effect.

The animal's death occurred at the 80th minute of blood exfusion, and the brain frontal lobe was immediately examined. The material for electron microscopy was fixed in 2% osmium acid on 0.1 molar phosphate buffer and on 3.5% glutaraldehyde on the same buffer. The preparation casting was carried out in the epoxy mass by conventional method. Ultrathin sections were made on the ultramicrotom "ЛКБ", and after contrasting with uranic acetate and lead, were examined with the electron microscope JEM-100B (Japan).

Verapamil represents an isochinoline derivative, while PV-88 is the acidic citrate (diethylamine) propyl 3 (n-fluorphenyl) 3 - oxipropel 1 pyrrol, where the fluorine is introduced into electronic acceptor domain. This original structural modification significantly increased Ca^{2+} slow channel blocker effect compared with its predecessor compounds. Both preparations block myocardial L channels, but they may also affect the CNS channels as well.

Results and discussion. Hypoxia in hemorrhagic shock causes significant morphological changes in the CNS cortex. All ultrastructural deviations are caused by discirculation. Morphological changes in the brain frontal lobe cortex develop only in acute ischemia. The cell intactness to a certain extent is maintained for a limited time interval. The reason of destruction, developed in the next period, is sharp decrease of pH and activation of lysosome ferments.

Our data show that rapid development of hemorrhagic shock causes sharp swelling and aggregation of mitochondria and cell chromatin marginalization, first of all, in the astrocytes (Fig .1), which indicates the start of the brain edema. These changes of all the blood erythrocytes point to the reaction of the brain peripheral blood vessels to decrease the circulated blood amount, caused by the blood capillary stasis (Fig .2).

In the hemorrhagic shock verapamil caused marked protective effect on the frontal lobe of the brain cortex. Astrocytes and neurocytes were mainly maintained. Mitochondria swelling was observed in some astrocytes. There were some vacuoles, indicating the start of mild brain edema (Fig .3).

PV -88 cerebral-protective effects are like the above-described effects of verapamil activity. In the animals, treated with verapamil and against the background of PV -88, practically intact astrocytes were observed. (Fig .4). Rarely there were observed singular vacuoles with electro-transparent mass, indicating mild edema of the brain.

PV-88 is Ca^{2+} slow channel blocker, characterized by cerebral-protecting effect as well, caused also by its ability to stop calcium ion damaging activity to cell membranes. This process was caused by hypoxia, the reason for which was a serious homodynamic disorder, therefore, such damage is characteristic of all parenchyma cells. If we consider the fact that calcium slow L channel blockers are characterized by hypotensive effect due to decrease of general peripheral resistance, then it could be used in pathologies, such as portal hypertension with encephalopathy. β adrenergic blockers are used in portal hypertension, but they have no cerebral-protective effect and belong to the agents affecting the vegetative nervous system. Verapamil and PV-88, similar to it, L are calcium slow channel blockers with hypotensive effect, in addition, they are characterized by cerebral-protective effects. Therefore, they can be used for liver severe damage of various etiology (viral hepatitis, cirrhosis, malignant growth, alcohol damage), also in portal hypertension with ammonia genesis encephalopathy. It should be noted that the preparation of this group nimodipin is used in subarchnoid hemorrhages. Their main pharmacodynamics is reduction of oxygen consumption. The hemorrhagic shock in cats is characterized by liver protecting effect of verapamil, the liver lobule cell structure is maintained. The membrane integrity is maintained, acini do not experience significant pathological changes, and the liver metabolism is significantly improved, manifested in obvious rearrangement of anaerobic respiration to aerobic one. These positive changes are more obviously manifested for PV-88 compared with the reference preparation verapamil. Verapamil and PV-88 as calcium slow L channel blocker may be effectively used for dementia prevention. It is known that among the causes of dementia the leading one is Alzheimer's disease (50-60% of cases) and cerebral vascular dementia (20-25%), yet acetylcholinesterase inhibitors used for its treatment have only an insignificant effect and represent palliative treatment only. Cognitive impairment in different parts of the brain is associated with â-amyloid deposit, which in turn, by acting on calcium channels, of membrane located in the plasma of

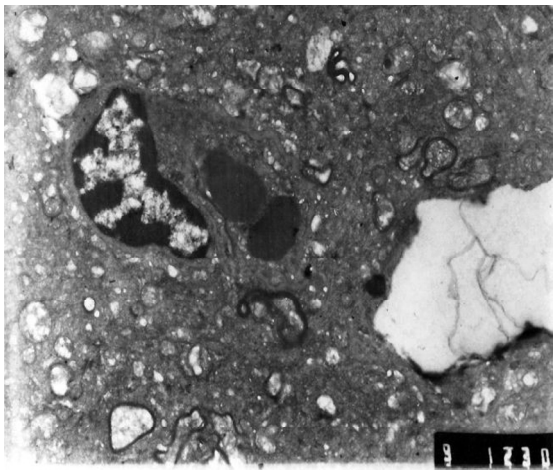

Fig. 1. Astrocyte of cerebral cortex. Swelling and aggregation of mitochondria and cell chromatin marginalization. Hemorragic shock. Electronogram x 12,000.

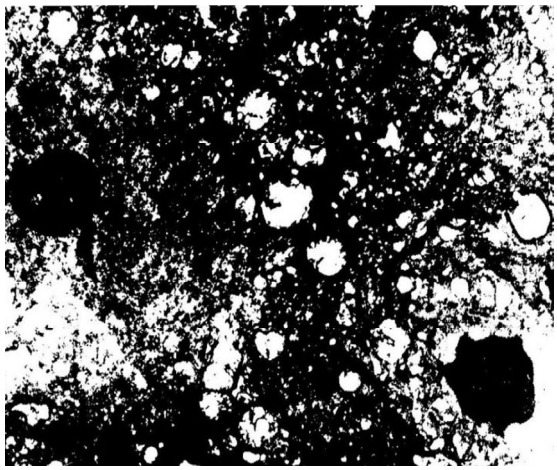

Fig. 2. Blood capillary of cerebral cortex. Blood capillary stasis. Start of the brain edema. Hemorragic shock. Electronogram x 12,000.

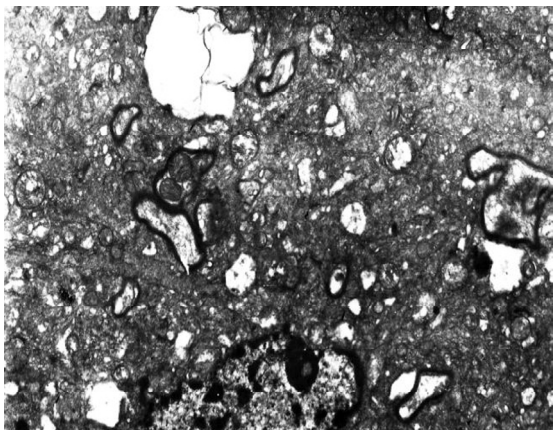

Fig. 3. Astrocyte of cerebral cortex. Hemorragic shock against the background of verapamil's action. Mitochondria swelling was observed in astrocyte. Electronogram x 13,000.

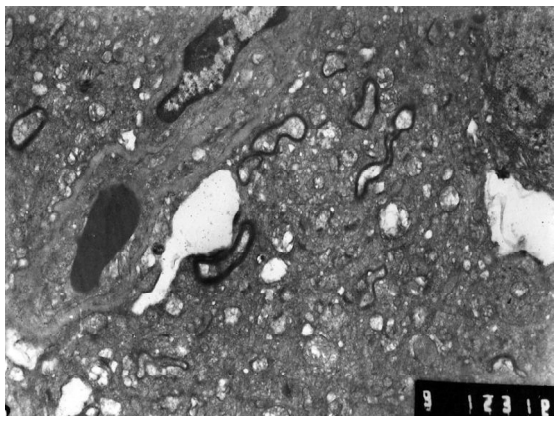

Fig. 4. Astrocyte of cerebral cortex. Hemorragic shock against the background of PV-88's action. Intact astrocytes were observed. Electronogram x 13,000.

somato-dendrites and axons, located in the cortex and hippocampus, causes its deregulation in cells. Studies conducted on animals have demonstrated that L-type calcium blockers weaken β-amyloid oligomer toxicity, tau protein destructive effect and improve autophagy. The L channel blocking and cerebral-protecting effect of PV-88 of the pyrrol group, studied by us, allows us to suppose its potential ability to block T channels as well. This requires further definition. Treatment of portal hypertension symptom (especially if it progresses with the picture of encephalopathy) by β blockers [6-14] which do not have cerebral-protecting effect and for β2 lysis properties in bronchial obstructive pathologies (bronchial asthma, atelectasis) cannot be used. In this regard, it is possible to use Ca^{2+} slow L channel blocker verapamil and other medications similar to it [15], and also PV-88, because at this time the frontal lobe of the brain cortex is significantly damaged [16,17], which requires further research.

Animal Handling. All experiments reported in this paper were carried out according to Institute National de la Santé et de la Recherche Medicale and Pasteur Institute animal welfare quidelines.

სამედიცინო მეცნიერებანი

პიროლის ზოგიერთი ნაწარმის მოქმედება თავის ტვინის ქერქზე ჰემორაგიული შოკის დროს კატებში

გრ. სულაბერიძე*, მ. ღვალაძე*, ე. ჭყონია*, თ. გეგეშიძე*, თ. ბუაჩიძე*, მ. ჩხაიძე*

** თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, დეპარტამენტები: ფუნქციური მორფოლოგიის; პათოლოგიისა და ციტოპათოლოგიის, სამედიცინო ფარმაკოლოგიის; ინფექციური დაავადებების; ფსიქიატრიისა და ნარკოლოგიის*

(წარმოდგენილია აკადემიკოს ფ.თოდუას მიერ)

ჰემორაგიული შოკის დროს განვითარებულ ჰიპოქსიაზე განსაკუთრებით რეაგირებს თავის ტვინის ქერქი, რომელშიც ადგილი აქვს დესტრუქციული პროცესების განვითარებას. პიროლის ჯგუფის ნაწარმი PV-88 ხასიათდება Ca^{2+} ნელი L არხის მაბლოკირებელი ეფექტით, ეს უკანასკნელი კი ქერქის უჯრედებს იცავს Ca^{2+} იონების გადატვირთვისაგან. ამიტომ შეიძლება ვივარაუდოთ, რომ ის მოქმედებს T არხებზეც.

ცდები ტარდებოდა მამრ კატებზე. სულ ჩატარებულ იქნა ექსპერიმენტის სამი სერია, თითოეულში 5 ცხოველი; I სერია – ჰემორაგიული შოკით (ჰშ); II სერია – ჰშ შესადარებელი ეტალონური პრეპარატ ვერაპამილის გამოყენების ფონზე და III სერია – ჰშ პიროლის ნაწარმებიდან PV-88-ის მოქმედების ფონზე.

ჰემორაგიული შოკის მოდელირება ხდებოდა საშუალო არტერიული წნევის (საწ) 40 mm/Hg-მდე თანდათანობით, ერთი საათის განმავლობაში დაქვეითებით. (ნარკოზი ნემბუტალი 40 მგ/კგ-წონაზე. სისხლის ექსფუზია შეადგენდა 40 მლ/კგ-ზე ხელოვნური სუნთქვის ფონზე 130-150 მლ/წთ-ში, არტერიული სისხლის pH შენარჩუნებული იყო 7.35-7.45, ხოლო PO_2 90 mm/Hg და მეტი. კატის სხეულის ტემპერატურის მუდმივობა შენარჩუნებული იყო 37.8-38.2°C-ზე. მარჯვენა ბარძაყის არტერიაში ინვაზიურად იზომებოდა საწ, მარცხნიდან – ხდებოდა სისხლის ექსფუზია, ხოლო გამოსაკვლევი პრეპარატები შეგვყავდა კატის მარჯვენა ბარძაყის ვენაში: ვერაპამილი დოზით 0.25 მგ/კგ-ზე და PV-88 3 მგ/კგ-ზე გახსნილი ფიზიოლოგიურ ხსნარში ამ უკანასკნელის მოცულობა არ აღემატებოდა გამოღებული სისხლის 1/20-ს.

ცხოველს ვაძინებდით სისხლის გამოშვებით და 1 სთ-ის შემდეგ ვიღებდით თავის ტვინის ქერქის მარცხენა შუბლის (Lobus frontalis) წილს ელექტრონული მიკროსკოპიისთვის სტანდარტული მეთოდით.

როგორც ჩვენი გამოკვლევიდან ირკვევა, მჟავე ციტრატს 1-3(დიეთილამინი პროპილ-3(n-ფტორფენილ-3-ოქსოპროპენ)პიროლი შიფრით PV-88-ს ახასიათებს ასტროციტებისა და ნეიროციტების დაცვის უნარი. მხოლოდ ასტროციტების გარკვეული ნაწილის მიტოქონდრიაში შეინიშნებოდა ვაკუოლები ელექტრონული-გამჭვირვალე მასის სახით და ის თავისი პროტექტორული ეფექტით მნიშვნელოვნად აღემატებოდა ეტალონურ პრეპარატ ვერაპამილს.

ყველა ექსპერიმენტი ცხოველებზე ჩატარებულია პასტერის ინსტიტუტის რეკომენდაციების გათვალისწინებით.

REFERENCES

1. *A. Biondi, G.K. Ricciardi, L. Ruybasset, et al.* (2004), AJNR, **25,** 6: 1067-76.
2. *C.M. Sayma, J.K. Liu, W.T. Couldwell* (2006), Neurosurg Focus, **21,** 3: E12.
3. *L.R. Marco-Contelles* (2011), Curr. Med. Chem., **18,** 4: 552-576.
4. *K. Ritchie, S. Lovestone* (2002), Lancet, **360,** 9347: 1750-1766.0020
5. *H. Abdali, R.F. Fard, M. Mahmoudi, et al*. (2007), JRMS, **12,** 6: 282-285.
6. *H. Al-Chamdi* (2011), Saudi J. Gastroenterol., **17,** 2: 155-158.
7. *W.S. Cho, H.S. Kang,J.E. Kim, et al*. (2011), Interv. Neuroradiol., **17,** 2: 169-78.
8. *H.J. Gelmers, K. Gortez, C.J. de Weerdt, et al.* (1988), Engl. J. Med., **318**: 203-207.
9. *J.D. Lefrandt, J. Heitmann, K. Sevre, et al*. (2001), Br. J. Clin. Pharmacol., **52,** 6: 687-692.
10. *M. Ghvaladze, T. Gegeshidze, Gr. Sulaberidze* (2010), Collected Papers Dedicated to the 80th Aniversary of TSMU, XLIV: 112-114 (in English).
11. *M. Ghvaladze, T. Gegeshidze, Gr. Sulaberidze, et al.* (2010), Science and Technologies, **7-9:** 117-121 (in Georgian).
12. *Gr. Sulaberidze, M. Ghvaladze, G. Didava, et al*. (2011), Georg. Med. News, **1,** 190: 65-69.
13. *R.J. Groszmann, G. Garcia-Tsao, J. Bosch, et al.* (2005), N. Engl. J. Med., **353**: 2254-2261.
14. *D. Tripathi, C. Graham, P.C. Hayes* (2007), Eur. J. Gastroenterol. Hepatol., **19,** 10: 835-845.
15. *S. Wolf, H. Martin, J.F. Landscheidt, et al.* (2010), Neurocrit Care, **12,** 3: 346-351.
16. *G.N. Kryzhanovskii, G.F. Leskova, V.I. Vdovichenko* (1996), Bull. Exp. Biol. Medic., **121,** 4: 352-355. DOI: 10.1007/BF0244
17. *G.F. Leskova, V.K. Lutsenko* (2002), Bull. Exp. Biol. Medic., **134,** 9: 263-266.

Received July, 2012