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EFFECTS OF NICOTINOIL L-PROLINE ON CEREBROCORTICAL MICROCIRCULATION NETWORK IN ACUTE CEREBRAL ISCHEMIA

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Abstract

Based on participation of neurochemical compounds of brain, including neuroactive amine acids in regulation of cerebral circulation, in our previous study it was discovered the stimulating effects of new synthetized dipeptide - nicotinoyl prolin on impaired after ischemia brain blood flow. It was demonstrated , that nicotinoyl prolin increases local cerebral circulation in brain cortex without any changes from systemic blood pressure.

The aim of presented study was to investigate the details of cerebrovascular activity of nicotinoyl prolin by evaluation of its effects on cerebrocortical microcirculation. In this purpose it was evaluated morphology changes on capillary network of brain cortex after acute ischemia, caused by left common carotid artery occlusion. Obtain results by non-injectional calcium adenosinetryphosphate method for evaluation of cerebrocortical capillary network density evident, that left common carotid artery occlusion is accompanied by noticeable decrease in mean diameter of all types of capillaries and the ratio of cortical network capillaries number with various diameter, which was prevented by intraperitoneal injection of nicotinoyl prolin in dose 10 mg/kg. Under the condition of acute disorder of cerebral blood circulation, caused by left common carotid artery occlusion, in animals treated by nicotinoyl prolin it was registered the increasing of mean diameter of all capillaries and number of functioning capillaries with simultaneous decreasing number of compressed and permeable only for plasma capillaries compare with ischemia.

Thus, obtained data indicated, that one of the possible ways of brain blood flow stimulation by nicotinoyl prolin could be its ability to improve the disturbed during acute ischemia cerebral microcirculation.

Keywords: *nicotinoyl L-proline, cerebral microcirculation, left carotid artery occlusion, acute cerebral ischemia*

Introduction

Despide the wide range of drugs for the correction of cerebral blood circulation, cerebrovascular disorders remain a leading cause of long-term disability, and the second leading cause of death worldwide [*Benjamin E et al., 2017; Feigin V et al., 2019*]. That's why the search for effective and safe drugs which are using for the treatment of cerebrovascular disorders, continues to be the one of main task of modern medicine.

Taking into account that among the activated

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complex processes that facilitate the restoration of an adequate blood flow in response of brain blood flow disorders the first response is the recovery of the cerebral blood flow through activation of the collateral circulation and anastomotic vessels, it became obvious that development of drugs with ability to improve cerebral microcirculation is very important for correction of impaired cerebral circulation [*Lapi D et al., 2015*].

Presented stady demonstreats the effect of new synthetised dipeptide Nicotinoyl L-proline on cerebrocortical microcirculatory network. Nicotinoyl L-proline is a dipeptide of nicotinic acid and proline. The both components of mentioned structure have a wide spectrum of biological activity.

Nicotinic acid is considered as a compound with a promising future and great attention especially in neurology [*Bodor E et al., 2008; Romani M et al., 2019*], based on ability to stimulate cerebral blood flow, reduce oxidative stress [*Chong Z et al., 2004*] and DNA damage,as well as its neurprotective property [*Kwon W et al., 2018; Valeria G et al.,2019*]. According to newly discovered mechanisms nicotinate participats in neurogenesis, stem cell differentiation, which is a most expressed in a case of formation of mature GABA-ergic neurons [*Griffin S et al., 2017*]

The neuroprotective activity of many peptide compounds determine with the presensce of proline residue in there structures [*Zhang Y et al., 2016*]. Thus, proline is a structural analoge of captopril, which is an inhibitor of angiotensin-converting enzyme, and possess wide range of pharmacological activity including neuroprotective, antihypertensive and angioprotective expressed during ischemic stroke [*Tao M et al., 2018*]. Glycyl-proline is also a proline-containing dipeptide with neuroprotective activity, which has an ability to stimulate brain tissue metabolism and prevent pathological shifts of neuroactive amino acids in a case of cerebral ischemia [*Bashun N et al., 2017*].

Based on above mentioned data the aim of presented investigation was study the influence of nicotinoyl L-proline dipeptide on cerebrocortical microcirculation in impaired cerebral blood flow.

Recent years more interest have small di- or tripeptides with natural origin which have been synthesized based on endogenic amino acids such as glycine, lisin, prolin etc. Peptides generally have very favorable pharmaceutical properties including high selectivity and potency for their target, minimal potential for drug-drug interactions, lack of accumulation in tissues, and effectively metabolized by endogenous enzymes to non-toxic metabolites [*Strand F 2003; Balasanyan M et al., 2015*]. Small peptides possess high cerebrovascular, antioxidant activity with an ability to regulate impaired cerebral tissue metabolism during ischemic injury [*Kanunikova N 2017*].

Compare with polypeptides of the same origin small peptides easily pass blood brain barrier, are more stable and peripheral activity is a less prominent [*Hökfelt T et al., 2003*]. Some of this kind of drugs such as noopept and picamilon have appeared high effectiveness for elimination of cerebrovascular disorders consequences [*Ostrovskaia R et al., 2002; Silkina I et al., 2005*]. Noopept is an esther of phenylacetyl and prolylglycin. In addition to pronounced anti-amnestic, neuroprotective, anxiolytic effects, it has the ability to stimulate cerebral blood circulation and correct the shifts caused by local cerebral ischemia, including the behavioral and morphological changes [*Balasanyan M et al., 2010; Amelin A et al., 2011*].

In our previous investigation it was demonstrated, that new synthetase dipeptide nicotinoyl L-proline stimulates the cortical circulation of brain in both cases acute ischemia caused by ligation of left common carotid artery and chronic ischemisation of brain tissue under the condition of movement restriction [*Aghamalyan I et al.,2020*]

The main purpose of presented research is an evaluation the effects of nicotinoyl L-proline on cerebrocortical microcirculation and microvascularisation network morphology changes in impaired cerebral circulation.

Materials and Methods

Experiments were carried out on inbred male white rats weighing 180-230g, kept at standard laboratory vivarium conditions according the Public Health Service Guide for the Care and Use of Laboratory Animals, approved by the Yerevan State Medical University Ethics Committee [*Garber JC, 2011*]. The day before the experiments animals were deprived of food with free access to water. Surgical interventions were performed under the general anesthesia by intraperitoneal in-

jection of 5% chloral hydrate with dose of 400 *mg/kg*.

Acute ischemia was modeled by common left carotid artery ligation. After ligation intraperitonealy was injected:

- in control group (n = 6) - 0.9% saline solution.
- in experimental group (n = 6) - nicotinoyl L-proline in dose 10 *mg/kg* dose

90 minutes after injection, when cerebrovascular effects of nicotinoil prolin riches its maximum value [*Aghamalyan I et al.,2020*], rats were decapitated. The brains were fixed in 5% formalin solution for further morphological staining.

Evaluation of the cerebrocortical capillary network density were performed using the non-injectional calcium adenosinetryphosphate method by Chilingaryan [*Chilingaryan A 1977; Chilingaryan A et al, 2006*]. This method is based on high selectivity of formed during ATP hydrolysis by calcium salts free inorganic phosphorus towards vascular tissue structures. Besides the detection of the vascular system from capillary network the method provides the opportunity to distinguish arteries from veins and capillaries [*Chilingaryan A, 1977; Chilingaryan A et al, 2006*]. After 1-3 days fixed brain was washed by running water for 30 minutes. The 90 μm thick longitudinal brain tissue slices received under the conditions of (-14 -16) °C by M25 cutter were subjected further process according to the requirements of the method. Fixed vascular network of the cerebral cortex in final preparations were examined by (Boeco BM-800) light microscope with (B-CAM 14MP) digital photo camera. Capillary diameter was measured with the B-Viwe program (Boeckel). The capillary diameter expressed by micrometers (μm) was determined as an average of 2-6 measurements. For the evaluation of microcirculatory network density 420 fields were observed.

The capillaries were distributed by mean diameter as follows: functional (4-10 *μm*), compressed (2.5-4.0 *μm*), permeable for blood plasma only (<2.5 *μm*). Obtained data were compared with same indicators of cerebrocortical capillary network of "Healthy, intact rats".

Statistical analysis was performed using the IBM_22.0.0SPSS statistical package (IBM, Armonk, NY, USA). Continuous variables with normal distribution were expressed as mean, standard deviation (SD), standard error (SE) and categorical variables presented as numbers and percentages. All p values were from 2-tailed tests, and results were deemed statistically significant at ($p<0.05$).

Result and Discussion

In carried out experiments it was observed totally 486 capillaries in control group with mean diameter 5.92±0,11*μm*., which was distributed as follow: number of functioning capillaries with diameter 6.1-10*μm* was 190 and with 4.1-6.0 *μm* was 181. The number of compressed capillaries with diameter 2.6-4.0 *μm* was 92 and number of capillaries permeable only for plasma with diameter less than 2.5 *μm* was 23.

Analysis of data obtained by microscopic observation evident that left carotid artery occlusion (LCAO) is accompanied by noticeable changes in mean diameter of all types of capillaries and the ratio of capillaries with various diameter of cortical network in both ipsilateral and contralateral hemispheres compared with control value which prevented by intraperitoneal injection of nicotinoyl L-proline in dose 10 *mg/kg* (Table 1).

Thus, left common carotid artery occlusion accompanied by decreasing of mean capillaries diameter for 45% and 41% in ipsilateral and contralateral hemispheres accordingly. Intraperitoneal injection of nicotinoyl prolin in dose10 mg/kg prevented the described deterioration, increasing the evaluated indicator for 53.84% and 43.8% in ipsilateral and contralateral hemispheres accordingly compare with ischemia.

After LCAO ischemic changes leads to reduction of functioning capillaries number (with diameter 6.1-10.0 μm from 39.1% till 7.1% in ipsilateral and 10.8% in contralateral hemispheres, which means, that compare with initial rate functioning capillaries was decreased 81.4% and 72.38% in ipsilateral and contralateral hemispheres accordingly. Injection of nicotinoyl L-proline immediately after ligation accompanied with noticeable prevention of ischemic changes, which appears by increasing of functioning capillaries with mentioned diameter more than 3.2 times in ipsilateral and for 51.85% in contralateral hemisphere compared with occlusion (Fig. 1).

Described above changes after LCAO was register for functioning capillaries with diameter 4.1-

Table 1.

Distribution and mean diameter of several type of capillaries after left common carotid artery occlusion and injection of nicotinoyl prolin in dose 10 *mg/kg.*

Capillaries type	Average diameter and number of capillaries				
	Control group	LCAO+saline		LCAO+NP (10 *mg/kg*)	
		LH	RH	LH	RH
All (0-10 *μm*)	5.92±0.11 (n=486)	3.25±0.08* (n=522)	3.49±0.09** (n=480)	5.0±0.09** (n=463)	5.02±015** (n=475)
Functional (6.1-10 *μm*)	8.57±0.08 (n=190)	7.60±0.19 (n=105)	7.83±0.13 (n=52)	7.86±0.11 (n=105)	8.54±0.10 (n=78)
Functional (4.1-6.0 *μm*)	5.06±0,04 (n=181)	5.14±0.06 (n=55)	5.09±0.07 (n=68)	5.06±0.04 (n=201)	5.04±0,04 (n=232)
Compressed (2.6-4 *μm*)	3.11±0.04 (n==92)	3.24±0.03 (n=161)	3.15±0.03 (n=171)	3.35±0.04 (n=111)	3.24±0.51 (n=113)
Permeable only for plasma (<2.5 *μm*)	2.09±0.09 (n=23)	2.05±0.02 (n=201)	2.01±0.03 (n=189)	2.14±0.03 (n=46)	1.97±0.06 (n=52)

Notes: ** - p <0.05 compared with control, ** - p <0.05 compared with LCAO, LCAO - left carotid artery occlusion, LH - left hemisphere, RH - right hemisphere*

6.0 *μm*: their number was decreased for 67.2% and 61.8% in ipsilateral and contralateral hemispheres accordingly. Effects of nicotinoyl L-proline on functioning capillaries with mentioned diameter expressed as for functioning capillaries with diameter 6.1-10 μm what is the evident with data obtained, that their number is increased for 3.55 times and 3.43 times in ipsilateral and contralateral hemispheres accordingly (Fig.2). After the ligation of carotid artery at 90-th min of occlusion it was registered increasing of compressed capillaries numbers in both hemispheres in the same mannier: till 35.9% in ipsilateral and till 35.6% in contralateral hemispheres. Observed elevation of compressed capillaries was prevented by nicotinoil prolin injection. Registered number of capillaries with diameter 2.6-4μm in brain cortex of treated rats was decreased more than 30% in both hemispheres (Fig.3).

It was established, then capillaries permeable only for plasma was more sensitive to the ischemic changes. Thus, their number after left common carotid artery occlusion was increased about 10 times in both hemispheres (from 4.7% in control till 44.8% and 39.4% in ipsilateral and contralateral hemispheres accordingly). It should be noted, that ischemic rat's treatment by investigated dipeptide contributed to the more pronounced decreased of

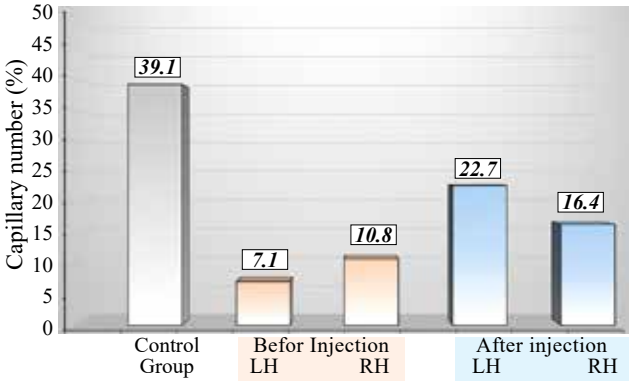

Figure 1. *Changes in number (in %) of functional capillars with diameter 6.1-10 μm in the cerebral cortex under the condition of left carotid artery occlusion before and after i/p. injection of nicotinoil prolin in dose 10 mg/kg.*

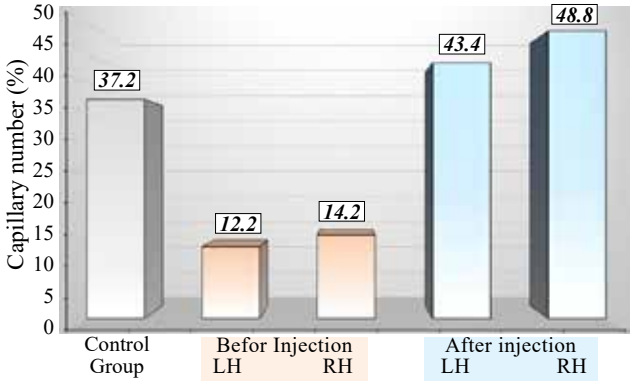

Figure 2. *Changes in number (in %) of functional capillars with diameter 4.1-6.0 μm in the cerebral cortex under the condition of left carotid artery occlusion before and after i/p. injection of nicotinoil prolin in dose 10 mg/kg.*

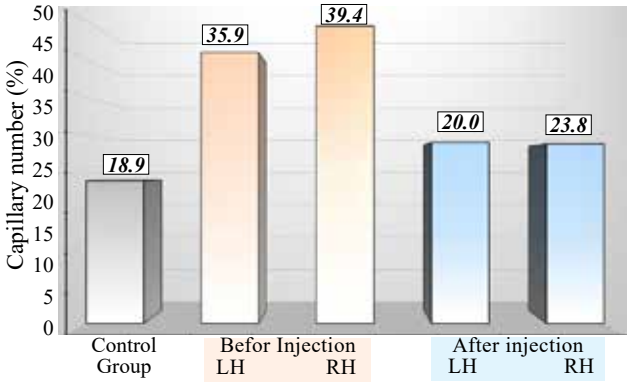

FIGURE 3. *Changes in number (in %) of functional capillars with diameter 2.6 - 4.0 μm in the cerebral cortex under the condition of left carotid artery occlusion before and after i/p. injection of nicotinoil prolin in dose 10 mg/kg.*

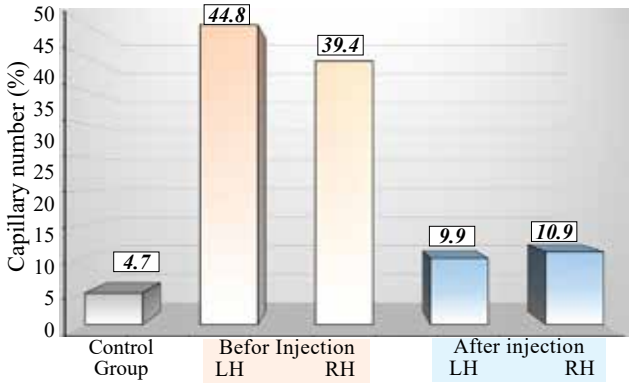

FIGURE 4. *Changes in number (in %) of functional capillars with diameter <2.5 μm in the cerebral cortex under the condition of left carotid artery occlusion before and after i/p. injection of nicotinoil prolin in dose 10 mg/kg.*

capillaries with diameter less than 2.5 μm, which numbers was decreased even for 4.5 times and 3.6 times compare with ischemic level in ipsilateral and contralateral hemispheres accordingly (Fig. 4).

Thus, presented data evident, that cardiovascular activity of nicotinoyl L-proline appears not only by stimulation of local cerebral blood flow as it was demonstrated before, but by its ability to activate the cortical microcirculation of brain through the increasing of functioning and decreasing of compressed capillaries after acute disorder of cerebral blood circulation, caused by left common carotid artery occlusion.

CONCLUSION

Conducted experiments conformed the cerebrovascular activity of nicotinoyl L-proline discovered in our laboratory before concerning the ability of investigated dipeptide to stimulate the local cortical brain blood flow in rats after acute ischemia [*Aghamalyan I et al, 2022*]. Obtained data provide one more evident for cerebrovascular activity of nicotinoyl L-proline based on its installed ability to stimulate the microvascular network in brain cortex by increasing the mean diameter of all capillary's types and number of functioning capillaries with decreasing of pressed and permeable only for plasma capillaries under the condition of acute cerebral ischemia.

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