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# Hypothalamic Proline Rich Peptide 1 (Galarmin) Protects Animals from Cardiac Infarction

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**Keywords:** *cardiac infarction, proline rich peptide, heart, TnI, isoproterenol, epinephrine*

**Introduction.** Discovery of hypothalamic neurosecretory nuclei (NSO and NPV) [1], the elucidation of vasopressine and oxytocin primary structures in fifties, as well as research related to the releasing hormones discovery [2] might be considered as a foundation for modern molecular neuroendocrinology. During the past few years, A.A. Galoyan with his collaborators added a new dimension into this area. Isolation and study of cardioactive neurohormones, as well as immunomodulators, such as new cytokines generating in the neurosecretory cells of the hypothalamus by A.A. Galoyan and co-workers [3] was a fundamental for the development of A.A. Galoyan's concept "Neuroendocrine cardiology and neuroendocrine immunology"[4]. "The most important A.A. Galoyan's concept is that the secreted peptides and cytokines act not only as a component of Endocrine System, but also as modulators of Immune System" (Abel Lajtha [4]).

In 1962-1964 A.A. Galoyan discovered neurosecretion of the cardioactive compounds of hypothalamus, which served as regulators of vessels' tonus of cardiovascular system [3, 5].

Isolation, identification of coronary dilatory active neurohormones - K, C, G, produced in hypothalamic neurosecretory nuclei NSO and NPV and their multiple forms as well as protein-hormonal complexes of the hypothalamus, released from brain into blood in physiological as well as in pathological conditions, especially during the infarction of the myocardium, points to the neurohormonal regulation

of cardiac circulation [6]. Moreover, neurohormone "C" appeared as a powerful medicinal compound for the treatment of experimental [7], clinical [8] acute as well as chronic types of the cardiac infarction.

In 1997 A.Galoyan and his co-workers discovered new hormonal system of the neurosecretory hypothalamic family of the proline rich polypeptidic (PRPs) hormones, isolated from the hypothalamic neurosecretory nuclei - NSO and NPV, which possessed wide range of biological activities [6]. PRP-1 possesses an anti-neurodegenerative properties in number of diseases including Alzheimer [9]. Powerful anti-neurodegenerative properties of Galarmin in aluminum dependant intoxication with the amyloidal polypeptides [9], spinal cord hemisection [10] as well as hemisection of the N. Ischiadicus [11] prompted us to assume that Galarmin might possess with the cardioprotective abilities also.

**Experimental techniques.** The models of myocardial ischemia. 1. Isoproterenol induced cardiac impairment. We have utilized in our experiments 150-160 grams rats, which were restricted from food for 24 hours prior to the experiments. Isoproterenol was injected twice intraperitoneally (I/P) 7mg/100 g with 24 hours time window in accordance with the method described by Genovese, A. et all [12].

The experiments were proceeded in accordance with the Animals Use Regulations of RA.

1. PRP-1 was injected I/P 2 hours before isoproterenol. Before terminal surgery and organ harvesting, animals were deeply anesthetized with 50 mg/kg of pentobarbital. Blood was withdrawn from the V. Jugularis by vacutiner with absorbed on the vial wall's heparin.

2. Epinephrine induced cardiac impairment. The model of cardiac impairment was established by single I/P injection of 0.4 mg/100 g in accordance to the protocol introduced by Cellarius, YG [13] and other authors [14].

3. Quantive evaluation of TnI in blood. The fluorimetric EIISA measurement of TnI (STRATUS CS analyzer; Dade Behring) excludes any experimental false, which might be caused by the presence of the serum hemoglobin (till 5g/l), cholesterin (till 10 g/l), bounded (till 0.4 g/l) and free bilirubin (till 0.2 g/l). Using lithium heparinate as an anticoagulant, blood samples were analyzed automatically. In accordance with techniques, after the first antibody was added to a glass fiber paper linked to the dendrimer, the sample and the second antibody were added. Finally, enzyme activity measurement was started by the substrate wash solution with the simultaneous washing of the unbound, labeled antibody. The antibodies, glass fiber paper, and substrate-wash solution are assembled into test packs, each containing all of the reagents for one test, which is ready and easy to use [15].

4. The statistical significance was determined by ANOVA-one way and the values were considered statistically significant if $p < 0.05$.

**Results and discussion.** The efficiency of PRP in the model of ephymephrine/ isoproterenol induced impairment of myocardium. We have chosen isoroterenol/ epinephrine induced cardiac impairment models for evaluation of PRP's effect. These models very well mimic the situation, when activity of the sympatric nervous system is elevated (during emotional stress, when there is an elevation of catecholamine in the blood stream, which induces damage of stomach, heart). The effect of epinephrine occurs through its influence on alpha as well as beta receptors. Isoproterenol is a synthetic derivative of catecholamine, which exclusively binds with beta-one and beta-two receptors [16].

Thus, by the comparison of double systems' results, we could propose the possible mechanism of direct or non-direct PRP-1 influence. The authors described the histological early stage heart destructions in these models. Microscopy investigations revealed myocardial cell lesions, which, according to the authors, could be distinguished as segmental and subsegmental contractures, intracellular myocytolysis, and primary granular disintegration and might remain in animal myocardium for many hours [13].

The level of the cardiac ischemia was determined by evaluation of the TnI level in blood. Cardiac TnI is a part of the Tn system and there are three tissue specific isoforms: cardiac, quick contractive and slow contractive muscle specific. It has molecular weight equal to 23 000 D [17]. The amino acid heteromorphism doesn't exceed more than 41 percentiles. Cardiac TnI has additional (from 26-32 dependant on the species) aminoacids that determines its high specificity as a marker for diagnosis of cardiac impairment [17]. Also, it is necessary to take into account, that TnI cannot be released during any skeletal muscles or renal pathologies [17]. The TnI is released into the blood stream during 4-6 hours after infarction. The TnI's detectable amount reaches its maximum after 18-24 hours and remains in the blood stream till seventh day [17]. The selective leakage of the TnI might occur due to apoptosis and necrosis in situation of acute severe ischemia in the acute coronary syndrome during less severe ischemia or even after exposure of myocardium to cytokines, such as a tumor necrosis factor alpha during unstable coronary heart disease (CHD) [17].

First, it was examined effect of PRP in Epinephrine-induced cardiac ischemia model (Fig. 1). The high efficiency of protection was notable after very low dose of PRP injection: 5-10 ug/100g prevented 90% of leakage of TnI in comparison with the positive control (single injection of Epinephrine) into the blood stream, whereas 12-18 ug/100 g of the animal had a vivid opposite effect, which was stimulation of cardiac markers outflux for 12-20%.

In isoproterenol model (Fig.2) of cardiac impairment, the trend of the protection/exacerbation was almost similar with the 18 ug/100 g turning point concen-

tration the injected PRP. The trend type was very similar in both models, however, the degrees of TnI leakage was higher in the Epinephrine model for 3 times. It might be explained by the fact that Epinephrine acts through alpha as well as beta-receptors, whereas isoproterenol acts only via the beta receptors. In addition, PRP clearly has a protective effect in both systems, which precludes the suggestion of its alpha or beta-receptor binding.

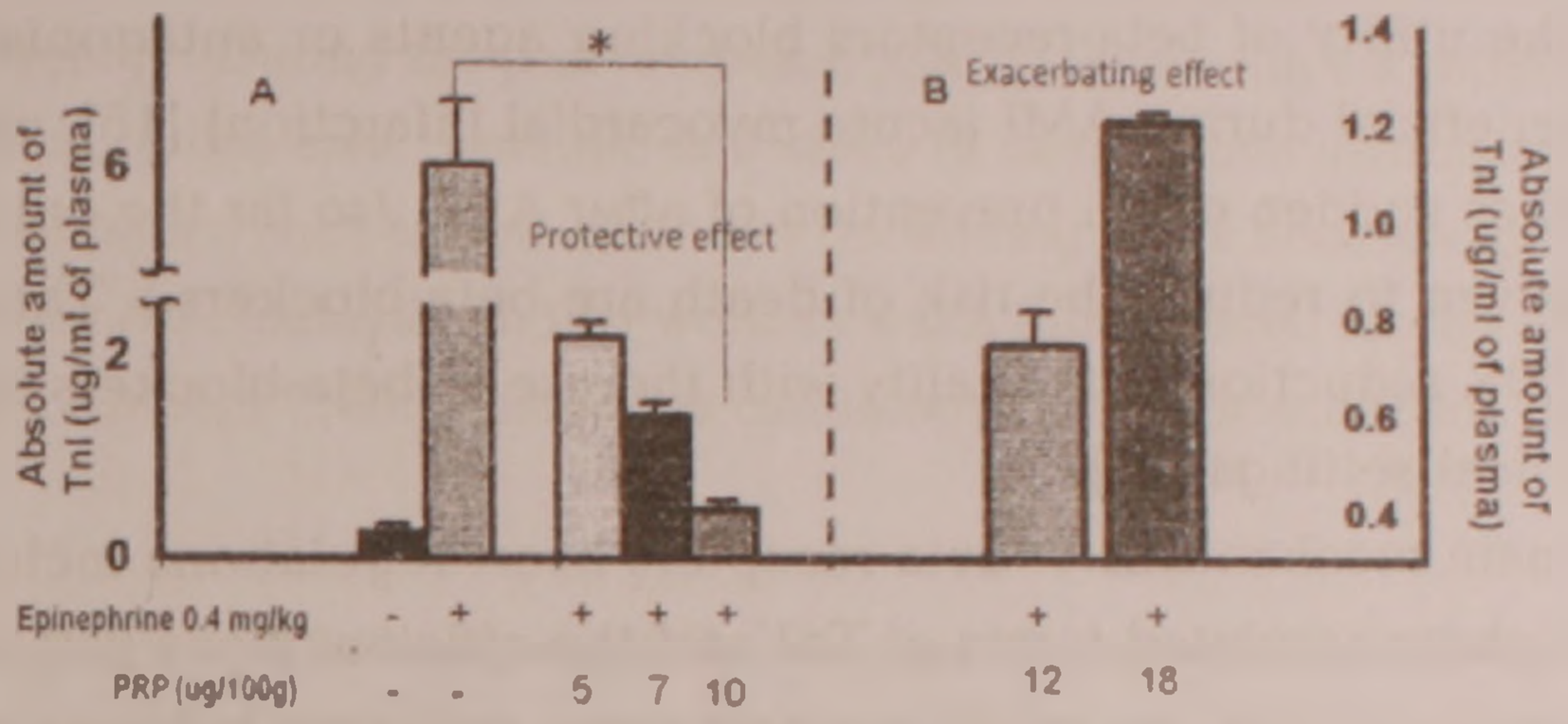


Fig.1. Cardio protective/deleterious effect of PRP, reflected by decrease of cTnI leakage into the blood stream in the model Epinephrine-induced cardiac impairment. A. In a dose dependant manner PRP was administered I/P 2 hours before the injection of isoproterenol and decreased the amount of cTnI in the blood stream. B. Increased dosage of PRP displayed reversed, deleterious effect and increased the amount of cTnI in blood ($n = 3$ rats per group; $p < 0.05$ between control and groups of the animals received PRP)

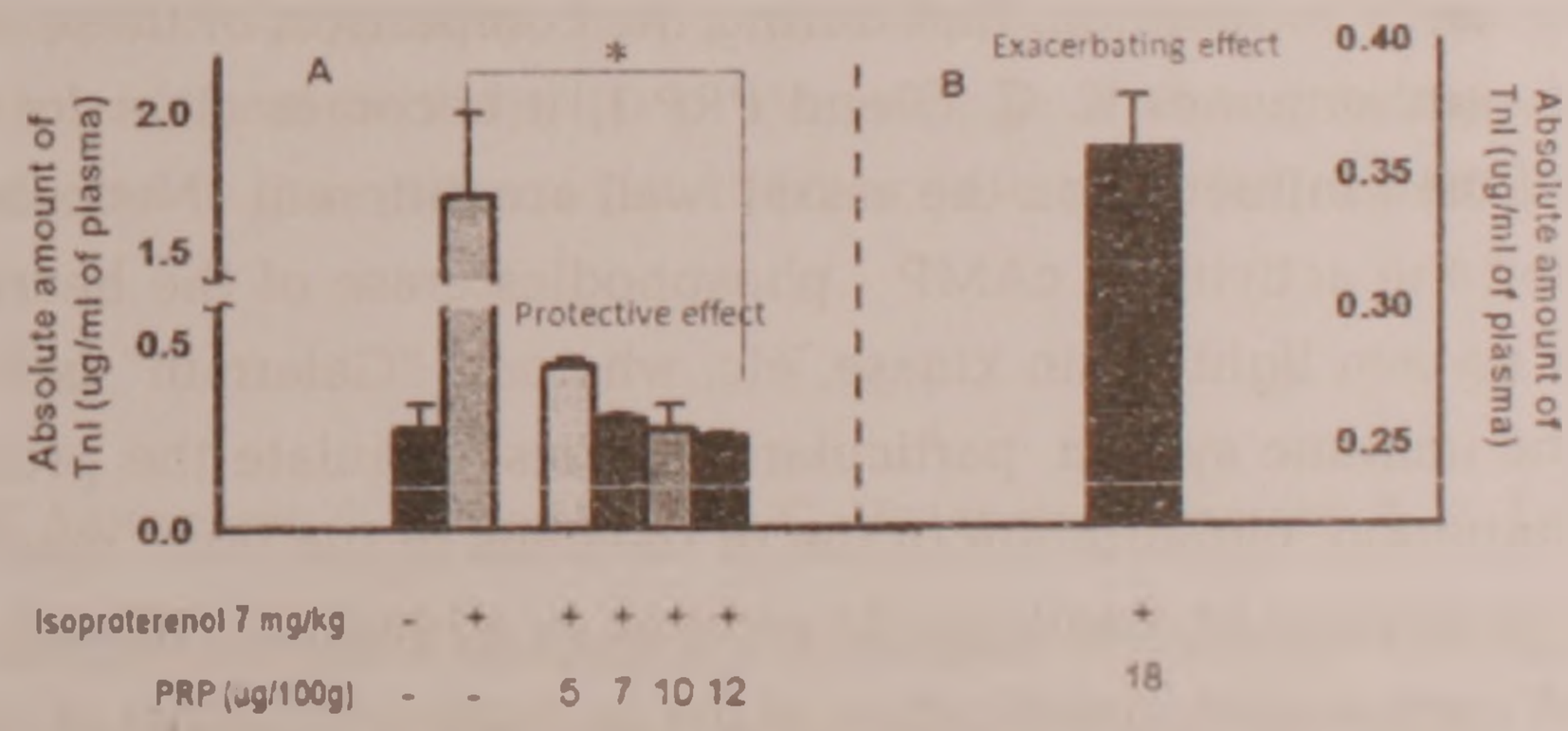


Fig.2. Cardio protective/deleterious effect of PRP, reflected by decrease of cTnI leakage into blood stream in the model Isoproterenol-induced cardiac impairment. A. In a dose dependant manner PRP I/P, which was administrated 2 hours before injection of Iso, decreased the amount of cTnI in blood stream. B. Increased dosage of PRP had a reversed, deleterious effect and increased the amount of cTnI in blood. Unless specified otherwise, the data are shown as a mean S.E.M. $n = 3$ rats per group; $p < 0.05$ between control and groups of the animals received PRP.

As a second negative control, we have injected PRP without administration of isoproterenol or epinephrine into the naive animals in a dose equal to 25 or

100 mg/kg of the animal's weight and monitored the leakage of TnI. The results of second negative control group were similar with the absolutely intact animals control groups (results are not shown).

Chronically increased effect of sympathetic nervous system on the diseased heart is mediated through beta-receptors in patients. This is manifested by elevated plasma level of Norepinephrine often before appreciable symptoms occur [18]. Thus, the utility of beta-receptors blocking agents or antagonist in clinical situation is beneficial during AMI (acute myocardial infarction) [18], chronic heart failure as well as sudden death prevention of after AMI, (so far the only drugs that have been proven to reduce the risk of death are beta-blockers.) There is an approximately 20% reduction in mortality with the use of beta-blockers after MI [18] and other clinical settings [18].

As the main mechanisms of beta-receptors heart regulations include involvement of (non)phosphorylated forms of TnI and the efficiency of PRP1 influence in this system occur via ARs, we might suggest some similarity between our peptide and clinically proved beta-blockers.

Taking into consideration the obtained data described above, it is possible to state that the neurosecretive hypothalamic nuclei produce not only cardiodilatory neurohormones (neurohormones K, C, G), but also cytokines- immunomodulators (proline rich polypeptides), which are able to regulate cardiac vessels, preventing vessels' spasm or be protective against cardiac infarction.

It is necessarily to mention, that during the comparison of these two types of compounds, neurohormones K, C, G and PRP-1, it becomes clear for us that the mechanisms of their influence on the vessel' wall are different. Neurohormones C, K and G inhibit the activity of cAMP - phosphodiesterase of the heart and brain, as well as the myosin light-chain kinase, etc, whereas, "Galarmin" is the powerful activator of the immune system, particularly it does stimulate the proliferation as well as differentiation, mobilization of the of the cells of the bone marrow.

The above mentioned results might serve as an addition for already performed and published comparative investigation of the immunomodulators of the neuroendocrine immune system of the brain in the light of the cardio-vascular system [4].

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Ք.Է. Դանիելյան, Ս.Գ Չաիլյան, Մ.Ս. Նազարյան, ակադեմիկոս Ա.Ա. Գալոյան

## Սրտամկանի ինֆարկտի արգելակումը կենդանիների մոտ հիպոթալամուսից սպացված պրոլինով հագեցած պեպտիդ-1-ի (գալարմինի) միջոցով

Հաշվի առնելով արտադրվող հիպոթալամուսի N. Supraopticus (NSO), N. Paraventricularis (NPV) կորիգներում Գալարմինի (ՊՀՊ-1) պաշտպանիչ հատկությունները նեյրոդեգեներատիվ ԿՆՀ պաթոլոգիաների ժամանակ, ուսումնասիրվեց վերջինիս հատկությունները իզոպրոթերենոլային եւ էպինեֆրինային սրտային ինֆարկտի մոդելների վրա կենդանիների մոտ:

Արտահայտված պաշտպանիչ հատկություններ նկատվեցին ՊՀՊ-ի ցածր դոզաների կիրառման պայմաններում (5-10 մկգ/100 գ. Կենդանիի քաշ). Հետաքրքիր էր. որ 18 մկգ/100 գ դոզան ցուցաբերում էր վատ բացասական էֆֆեկտ, խթանելով TnI-ի արտազատումը 12-20%-ով.

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## Предотвращение инфаркта миокарда у животных с помощью богатого пролином пептида-1 (галармина), выделенного из гипоталамуса

С учетом протекторного влияния галармина, продуцируемого в N. Supraopticus, N. Paraventricularis гипоталамуса при нейродегенеративной патологии ЦНС, изучено действие богатого пролином пептида-1 на изопротереноловой и эпинефриновых моделях повреждения миокарда у животных. Высокая степень протекции отмечена при инъекции низких доз препарата (5-10 мкг/100 г массы животного). Интересно, что доза, равная 18 мкг/100 г пептида, имела ярко выраженное отрицательное влияние, стимулируя дополнительный выброс TnI на 12-20%.

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