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IMMUNOMODULATORS OF THE HYPOTHALAMUS: PRIMARY STRUCTURE AND ROLE IN SIGNAL TRANSDUCTION AND SMOOTH MUSCLE CONTRACTILITY

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From the composition of low molecular weight proteins of hypothalamic peptides (HP) obtained by methods (Galoyan A.A., 1973) using continual electrophoresis (Elfer-21) and HPLC on reverse-phases we isolated 117 polypeptides and proteins-regulators and chemical messengers of immune cells. Several neuropeptides modulate antibodies formation in inductive and productive periods of immune response to polyclonal (LPS) T-dependent (SRBC), T-independent (DNP-ficol) antigens in vitro and in vivo (V.Aprikian, K.Galoyan, 1993). It was found that HP were capable to modulate the antibody formation. Thus, HP modulate the antibody genesis.

We isolated several immunomodulators of brain (from hypothalamus) and the primary structure tentatively was discovered. Among them immunophilin was discovered – a receptor of immunosuppressor-FK-506. The complete immunosuppressor FK-506 with immunophilin inhibited the activity of calcineurin – playing a role in transduction processes in T-cells.

Taking into consideration the existence of IL-1, IL-2 etc. as well as several immunomodulators of brain discovered by us, I believe that the cells of magnocellular and parvocellular nuclei of hypothalamus can fulfil the role of T- and B-like cells in the brain.

The chemical messengers, immunomodulators, produced by hypothalamic cells, are factors for the protection of brain against infections and damages. The presence of immunomodulators of brain as well as peptidergic nerve fiber and neuroendocrine cells in lymphoid tissue including mucous – associated lymphoid tissue, suggests a potential structural link between the nervous and immune systems.

1. Isolation of Thymosin β_1 from the Hypothalamus

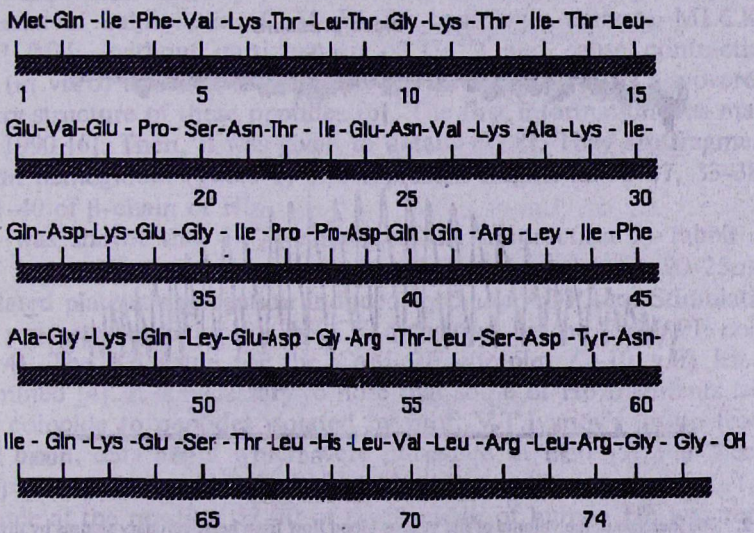
A new endogenous antagonist of CaM thymosin β_1($T\beta_1$) is a smooth muscle relaxant. CaM-binding proteins and peptides were isolated from protein-peptide fraction (PPF) of hypothalamus and purified to

homogeneity by reverse-phase HPLC. The interaction of $T\beta_1$ with CaM was analyzed by the affinity HPLC [1]. PDE and CaM were isolated from bovine brain by the affinity HPLC method [2]. The activity of PDE was assayed by the modified methods described [3].

It was discovered in bovine hypothalamus a neuropeptide molecule which was identified as (1-74) $T\beta_1$ by electrospray mass-spectral and micro-sequence analysis (Table 1). It was established that $T\beta_1$ at calcium concentration of 100 μM can be coupled to CaM. In the presence of saturating concentration of calcium $T\beta_1$ binds to CaM-Biopore HPLC column (4.6 x 250 *mm*) is eluted by the addition of EGTA or W-7. Only triton X100 solutions at the highest concentrations (>1%) eluted peptide. The results indicated that $T\beta_1$ would not bind to the PDE-Biopore HPLC column in the presence or absence of calcium. To study the nature of CaM-binding to immobilized PDE in the presence $T\beta_1$ we determined the ability of this peptide to elute CaM from the PDE-Biopore HPLC column (4.6 x 250 *mm*). $T\beta_1$ in the concentration of 10 *nM* and 100 *nM* completely eluted CaM from the column with immobilized PDE. During reversed-phase rechromatography (Biopore C18 Si300 10 μ4x250 *mm* HPLC column) of the eluted sample of CaM $T\beta_1$, complex two peaks were isolated corresponding to the peaks of CaM and $T\beta_1$. The effect of $T\beta_1$ to bind to CaM was accompanied by the strong inhibition of CaM - stimulated PDE activity (Ki = 15 *nM*) hydrolysis. The Hill analysis (0=4) demonstrated the strong positive cooperativity in $T\beta_1$-CaM complex formation. The 60-fold increase of the constant for PDE activation by CaM (2 *nM*) was accompanied by a significant decrease in maximum rate of cAMP hydrolysis (Fig.1).

Table 1

The primary structure of coronary dilatory polypeptide of hypothalamus (Thymosin β_1)



The results obtained showed that $T\beta_1$ caused the increase in volume of the venous blood flow from heart coronary sinuses by 90% or more after intravenous administration in a dose 100 *nMol/kg* without changes in blood pressure (Fig.2). The coronary dilatatory activity of $T\beta_1$ correlated well with the inhibition in a dose-dependent manner of human platelet aggregation induced by ADP (5 *mM*) as shown in Fig.3. Thus, there were discovered new properties of $T\beta_1$. It was indicated the structural-functional high affinity of $T\beta_1$ CaM. It was suggested that $T\beta_1$ may regulate CaM level and myosin light chain phosphorylization due to its ability to act as a natural antagonist of CaM under condition of high concentration of calcium in a cell.

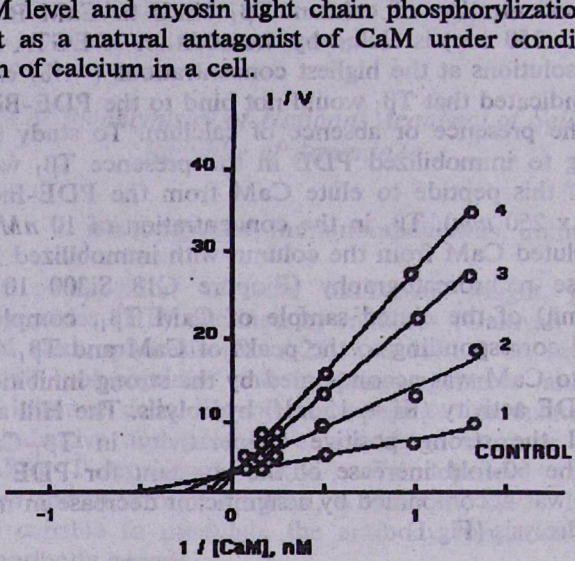


Figure 1. Inhibition of CaM-stimulated activity of PDE by Thymosin b1. Purified to homogeneity PDE was assayed at 30°C with 0.5 *μM* [^{3}H] cAMP, 5 *μM* MCl_2, 2 *μM* $CaCl_2$ and indicated concentrations of CaM or abscissa with 10 *nM* (2), 100 *nM*(3), or 1000 *nM* (4) of $T\beta_1$.

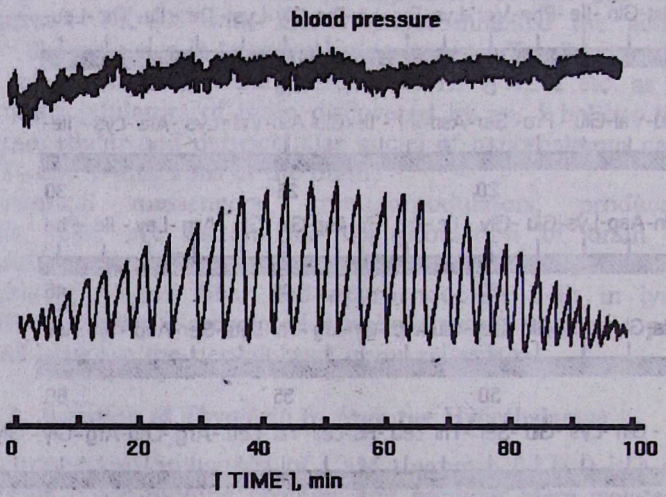


Figure 2. The increase of the volume of the venous blood flow from heart coronary sinuses by thymosin β after intravenous administration in a dose of 100 *nmol/kg*.

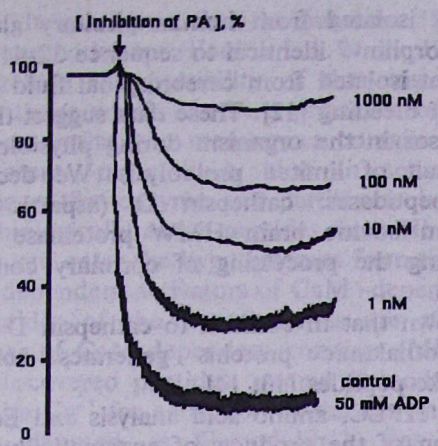


Figure 3. Inhibition by $T\beta_1$ of human platelet aggregation. Effect of $T\beta_1$ was examined in human platelet aggregation by 5 *μM* ADP. The human platelets were preincubated without (control) or with $T\beta_1$ at 37°C with stirring for 3 *min* in the aggregometer cuvete before stimulation.

2. Discovery of Ca^{2+}-CaM-Replacing Peptide Systems of Hypothalamus (CCRPS)

a) Calmodulin-binding Ca^{2+}-independent activators of Ca-dependent enzymes:

In 1988 five polypeptides with coronary constrictory properties were isolated from bovine hypothalamus protein-peptide reaction described by me (unpublished data), using gel filtration of Sephadex G-10, ion-exchange chromatography on Dowex 50*W*×8, semi-preparative and analytical reverse-phase HPLC [4]. For these groups of neuropeptides calmodulin is a target protein [5]. They antagonize with monospecific anti- calmodulin antibody for calmodulin binding, although they are not fragments of calmodulin. CaM F1-F5 complexes activate MLCK and cAMP PDE (without participation of Ca^{2+}) and cause contraction of aorta (in vitro) and coronary vessels (in vivo).In 1990 we discovered the primary structure of these peptides [6]. The first information was made by us in 1990 [6]. Then, it was given in details [7, 8]. They are fragments of β-chain hemoglobin (Table 2) and represent fragments 33-37, 33-38, 32-39, 31-40 of β-chain of Hb.

It was shown that PF_{1-5} enhanced the contraction in rabbit aortic strips in a K^+-depolarized mixture (approximately to 20-25*μ*) and stimulated platelet aggregation induced by 5 *μM* ADP too. Stimulation of these processes accounts for MLCK activation by the CaM-PF complex (Fig. 4). The Kd value for the CaM-PF complex (2-10 *nM*) has been determined [4]. It is necessary to note that some of Hb fragments isolated by us coincide to peptides isolated by prof. V.T.Ivanov's group from the whole brain, data about which were published in 1991 [9, 10]. Recently, an opioid peptide called LVV-hemorphin-6, corresponding to the sequence at the position 32-40 of the β-chain of human Hb was reported to inhibit the angiotensin converting enzyme activity [11]. This peptide

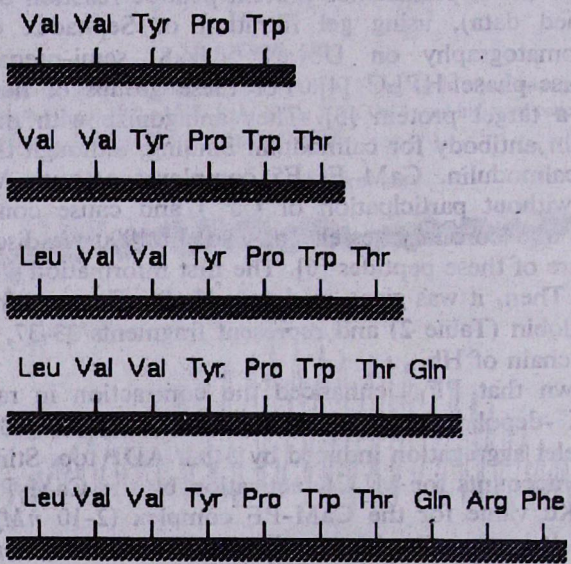
has been originally isolated from human pituitary gland. The peptide designed LVV-hemorphin-7 identical to sequence 32-41 of the β-chain of human Hb has been isolated from cerebrospinal fluid (CSF) of patients with cerebrovascular bleeding [12]. These data suggest that Hb or Hb-like protein is a precursor in the organism during physiological pathological processes as a result of limited proteolysis. We decided to use two intracellular endopeptidases: cathepsin D (aspartic proteinase) and isolated by us from bovine brain HMW proteinase (cathepsin E-like enzyme) for studying the processing of coronary constrictory peptides from β-chain of Hb.

It has been shown that in contrast to cathepsin D HMW proteinase from bovine hypothalamic proteins generates coronary dilatatory neurohormone C-like peptides [13, 14].

Recently, using HPLC, amino acid analysis and Edman degradation for the identification of the products of enzymatic hydrolysis, we have found that HMW proteinase (but not cathepsin D) generates the coronary constrictory peptide or LVV- hemorphin-7, which corresponds to the sequence at position 31-40 of the β-chain of bovine Hb (residue 32-41 of the β-chain of human Hb) by cleavage of the Leu^{30}-Leu^{31} and Phe^{40}-Phe^{41} bonds of the β-chain of bovine globin [14].

Table 2

Calmodulin-binding coronary constrictory peptides of hypothalamus



Thus, data obtained give us reason to suppose that Hb itself or Hb-like protein is the precursor of the coronary constrictory peptide, which can be formed in the organism during physiological or pathophysiological processes as a result of limited proteolysis of this protein by HMW

aspartic proteinase. It was shown that the fragment 32-37 of hemoglobin β-chain is an immunomodulator (Fonina L.A. et al., 1991).

We cannot exclude the possibility of the existence of a Hb-like protein in nervous tissue which can be a precursor of these peptides. At the same time since erythrocyte membranes contain the HMW aspartic proteins [4], it seems more likely that in some physiological or pathological processes this enzyme can participate in processing of the above mentioned biologically active peptides from Hb. We don't exclude that some peptides can enter the hypothalamus from the blood.

b) Ca-CaM-independent activators of CaM -dependent enzymes.

Three polypeptides of the hypothalamus are stimulators of basal activity of a number of CaM-dependent enzymes (MLCK, cAMP PDE, etc.). The newly discovered peptides, named C-modulins in contrast to CaM did not require Ca^{2+} for activation of the enzymes [15, 16]. One of these stimulators, denoted as C-modulin-3, has been purified to homogeneity by reverse phase HPLC and tentatively identified as thymosin β_4 (1-39) by mass-spectrometry and Edman microsequence analysis [17] (Table 3). We demonstrated the influence on basal activity of rabbit skeletal muscle MLCK, in comparison with that of thymosin a1 and thymosin b4 (16-18, 11-19, 25-31) as well as on the CaM-dependent PDE. C-modulin-3 is a very potent stimulator of MLCK and cAMP PDE without participation of Ca^{2+} and CaM (Fig. 5).

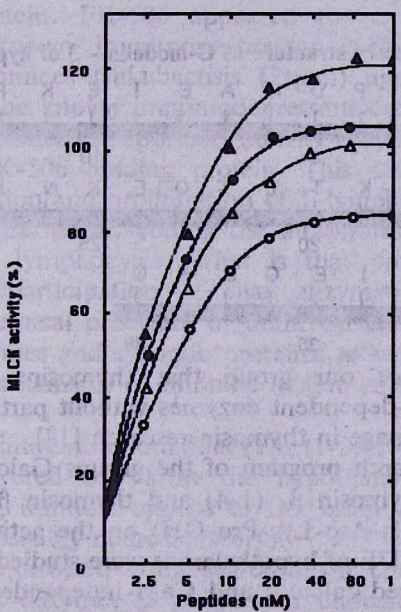


Figure 4. Ca^{2+}- independent activation of myosin light chain kinase by hypothalamic coronary constrictory peptide factors (PF3-R and PF4-x) synthetic peptides (P2-° and P3-•).The amount of enzymes producing 50% phosphorylation of myosin light chains at 25°C for 25 *min* is taken as 100% MLCK activity.

From the hypothalamus it was isolated also C-modulin-2 and its primary structure was determined: Glu-Lys-Ala-Gln-Gly-His-Pro-Gln-

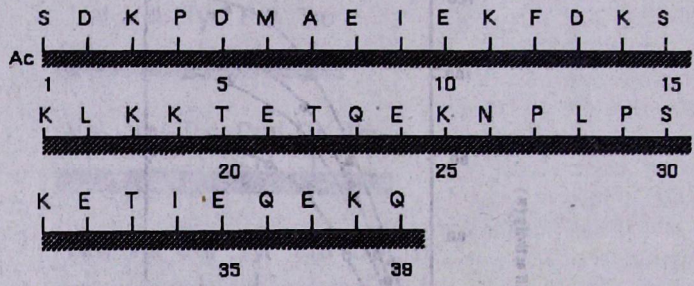
Asp-Gly-Asn-Pro-Val (Galoyan A.A., Gurvits B.Ya., 1991). It was a fragment of Myelin Basic Protein (MBP).

Of particular interest is the finding that each of the indicated enzymes, being CaM+Ca^{2+}-dependent, possesses sensitivity to activation by C-modulins, comprises a system of universal Ca^{2+}-independent enzyme regulators. They are calcium-calmodulin replacing peptide systems of brain activators of Calmodulin-dependent enzymes. More recently a number of other CaM-dependent enzymes - phosphorylase kinase and CaM- dependent protein kinase – were also found by us to be affected by C-modulins in cooperation with Dr. Livanova et al. from prof. B.Poglasov's laboratory (unpublished results).

Although a great deal is known at the molecular level about Ca^{2+}-dependent interactions of calmodulin with other molecules, such as peptides and enzymes, much less is known about the physiological significance of these interactions. It was not known whether calmodulin is the only ubiquitous regulator of a variety of eukariotic processes, including muscle contraction, cell division, cyclic nucleotide metabolism and glycogen metabolism, attributed to changes in the intracellular concentration of Ca^{2+}. The presence of Ca^{2+}-independent regulators of the so-called CaM-dependent enzymes may cause an ambiguity in the study of CaM-mediated processes [17, 18].

Table 3

The primary structure of C-modulin - 3 of hypothalamus



The findings of our group that thymosins act as C-modulins, regulators of CaM-dependent enzymes without participation of Ca^{2+} and CaM, open a new page in thymosin research [18].

In a joint research program of the groups Galoyan/Volter the effect of fragments of thymosin β_4 (1-4) and thymosin β_9 (1-4) (Ac-Ser-Asp-Lys-Pro and Ac-Ala-Asp-Lys-Pro-OH) on the activity of CaM-sensitive cyclic nucleotide PDE of hypothalamus were studied recently [19].

It was discovered calcium and CaM-independent inhibition of PDE by thymosin β_4 (1-4) and thymosin β_9 fragments. A tetrapeptide Ac-Ser-Asp-Lys-Pro-OH was isolated from calf bone marrow, possessing the ability to inhibit proliferation of hematopoietic pluripotent stem cells [20]. The tetrapeptide appeared to be identical to the aminoterminal part of thymosin β_4. It cannot be excluded that thymosin β_4 is a precursor of this tetrapeptide. In mammalian tissues thymosin β_4 is very often associated

with another highly homologous peptide of the thymosin family, thymosin β_9, as well as with modulators of immunity and neuroendocrine ciruits [21]. However, the fundamental mechanisms of actions of thymosins and their fragments remain to be resolved. Our suggestion is that the tetrapeptide realizes its effects on lymphocytes stem cells by the mechanism of Ca^{2+}-independent inhibition of cAMP-PDE. The results obtained by us have shown a specific function of Ac-Ser-Asp-Lys-Pro-OH as a releasing factor promoting an entry of coronarodilatory neuropeptides, previously discovered by us in hypothalamus [22, 23], into the general circulation, probably, through changes in the cyclic nucleotide level in the brain (Galoyan A.A., Alexanian, in press).

3. Discovery of immunophilin (FK-BP) in the hypothalamus

In the composition of Ca-CaM replacing peptide systems of brain (coronary constrictory peptides) we were able to isolate new protein(s) and primary structure was determined [24, 25] (Table 4). The search of the corresponding structure according to the bank of peptides showed that the deciphered sequence belongs to the bank of immunosuppressor FK-506 binding, possessing enzymatic activity of peptidyl-prolyl-cis-trans-isomerase. The data of amino-acid analysis together with Mr=11778.4D obtained on the basis of mass-spectra demonstrated full coincidence of the isolated polypeptide structure with the one of the native molecule of FK-506 binding protein. FK-506 appeared to be 10-100 fold more effective than the known immunosuppressor cyclosporin A. FK-506 isolated from streptomices tsukubaensis (Japan) appeared 10-100 fold more effective than the known immunodepressant cyclosporin A. By the way, this compound possesses suppresser activity only in the complex with the specific to it FK-506 binding protein. This activity is noticed in inhibition, differentiation and proliferation of T-lymphocytes as a result of blocking the gen transcription, coding the biosynthesis of interleukin-2, growth factor of T- lymphocytes. What is that protein doing in the hypothalamus? The participation of that enzymatic protein can be supposed in neurochemical processes of brain via changes of conformation of different enzymes and synaptic proteins, as well as in the formation of regulatory mechanisms of immune system of the brain itself and whole organism.

A number of the immunomodulators ($T\beta_4$ (1-39), $T\beta_1$, etc.) as well as immunophilins discovered by us in the hypothalamus testify to the existence of a strong immune system of the brain itself, and namely, hypothalamic cells (probably neurosecretory).

Our work must be directed to the search of the endogenous activators and inhibitors of that enzyme, as well as for understanding the role of these proteins in the process of transduction in nervous system.

Besides participation of that enzyme in the isomerization of Ca-dependent enzymes, it can participate in Ca metabolism, as calcitonin is one of the substrates of FK-506 BP. Moreover, the complexes of FK-506 with FK -BP and cyclosporin A with cyclophillins participate in Ca metabolism and protein phosphorylation. Taking into consideration the

existence of Iph(s) in the neurosecretory areas of hypothalamus, I believe that the multiple forms of Iph(s) probably are synthesized by hypothalamic neurosecretory nuclei simultaneously with the known neuropeptides produced by these cells Vasopressin, Oxytocin, cardioactive hormones etc., playing an important role for the folding as well as for the regulation of Ca^{2+} metabolism in the sarcoplasmic and endoplasmic reticulum. I think there are endogenous activators and inhibitors of Iph(s), including thymosin β_4 (1-39), β_1, β_9. In other words in the brain there are systems of activation and inhibition of immune system. The data obtained by us indicate that Iph(s) may take part in the regulation of metabolism of the secondary messengers (Ca^{2+}, cyclic nucleotides) in the brain. Iph(s) can participate in the mechanisms of transduction in the neurosecretory neurons of hypothalamus.

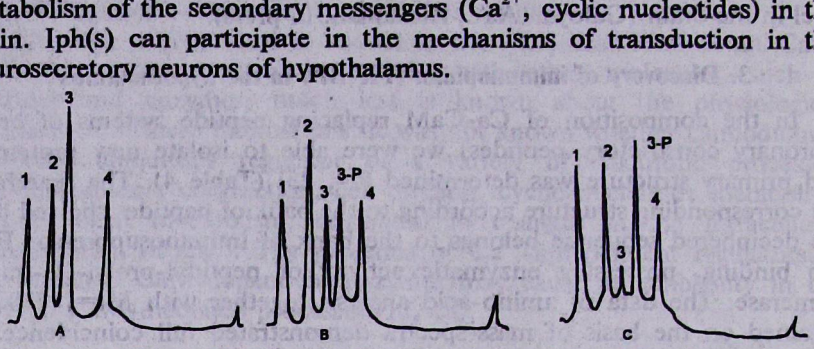


Figure 5. Phosporylation of LC_2 of a representative myosin preparation. Myosin was incubated with myosin light chain kinase in the absence (A,C) or in the presence (B) of calmodulin. Incubation mixture contained: no effector (A),C-modulin-3 (C) at concentration 50 *pM*

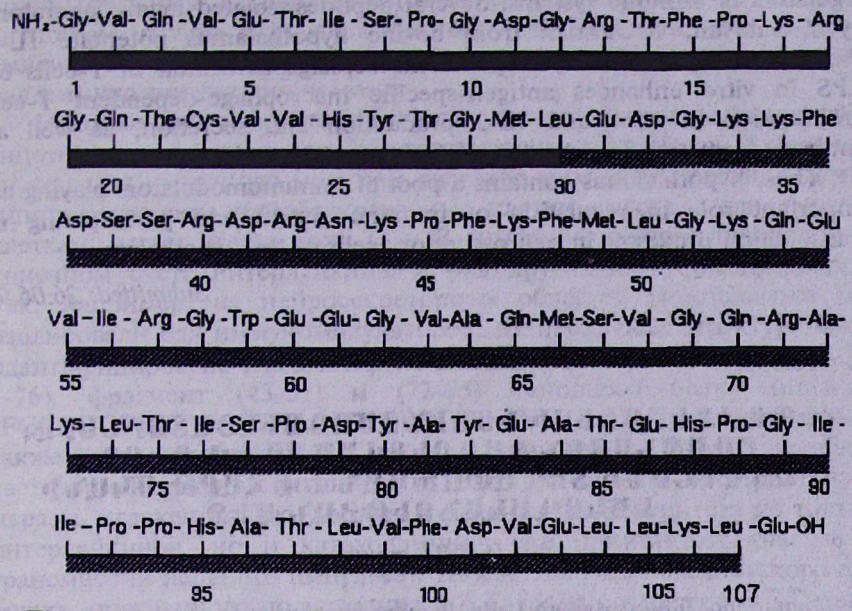
From the data obtained by the mechanism of action of the cardioactive neuropeptides of the hypothalamus we can conclude the following: the relaxants of the coronary vessels (NC,NG) are the inhibitors of both cAMP PDE and MLCK, as well as of Ca-binding protein and antigens of CaM. The increase of the level of cyclic nucleotides is a relaxation factor [26].

Neurohormone "C" inhibits MLCK and prevents the phosphorylation of the light chain of myosin. Moreover, NC decreasing the level of Ca ions till 10^{-7}-10^{-8} in the cytoplasm, is switching off the mechanism of Ca^{2+}-CaM MLCK complete formation [27]. Step by step involving this mechanism, NC has a rather positive action during myocardial infarction as well as pancreatitis and pancreonecrosis, having angiogenic role, restoring necrotic vessels of the pancreas. At the same time by their biological effect and by their physico-chemical peculiarities NC and NG differ from those of NK.

On the contrary, the nanomolar concentration of hypothalamic coronary constrictory neuropeptides is a powerful activator of cAMP PDE in one case without Ca^{2+} and CaM in the other, case by binding with CaM without Ca^{2+}-ions participation.

Table 4

The primary structure of FS-506 binding protein of brain (peptidyl-prolyl-cis-trans-isomerase)



These two groups of peptides activate MLCK and increase phosphorylation of MLC. These two mechanisms are enough for the heart smooth muscle contraction. Thus, new systems of neuropeptides intracellular regulators of secondary messengers metabolism of the smooth muscle contraction were discovered.

It is of a special interest the participation of thymosin β_4 (1-39) and $T\beta_1$ in the relaxation-contraction cycle of the smooth muscle.

It is of a great biological interest the problem concerning the conformational changes that calmodulin undergoes when bound with peptides F1-F5.

The fact that the new peptide system have a regulatory influence on Ca^{2+}-CaM activated enzymes without Ca ions and CaM participation (C-modulins) does not mean that the role of Ca ions in these processes is excluded. On the contrary, the concentration of Ca^{2+} in the cell determines the shift of one type of regulation (with Ca ions participation) into another, without Ca ions.

It is very important, however, to detect the concentration of Ca ions in the organism which are responsible to this shifting.

The results of our investigations testify that there is a number of alternative paths of regulation of both Ca^{2+}-CaM activated enzymes, heart activity and smooth muscle contractility. In this Ca^{2+}-CaM replacing peptide system an important role belongs to C-modulin-3, thymosin β_4, β_1, coronary dilatatory neurohormones (Ca ions translocators), hemoglobin and different fragments of $T\beta_1$ and MBP, as well as a new Ca-binding protein and ubiquitin (1-74).

Many of these peptides are the regulators of the cardiac circulation and chemical factors for integration of the functional system in the endocrine hypothalamus-neuroendocrine heart (22, 26, 27), as well as regulators of immune systems. Several peptides isolated in my laboratory by K.Galoyan, R.Galstian from bovine hypothalamus potentate IL-1 production, and the early stage of macrophage activation of T-cells by LPS in vitro enhances antigen-specific macrophage-dependent T-cell proliferation in vitro and IL-2 production and secretion, as well as antibody formation (V.Aprikian, K.Galoyan, in press).

Thus, hypothalamus contains a pool of immunomodulators playing an important role in regulation of immune system and participating in transduction processes in neurosecretory cells of the hypothalamus.

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ՀԻՊՈԹԱԼԱՄՈՒՍԻ ԻՄՈՒՆՈՄՈԴՈՒԼՅԱՏՈՐՆԵՐԸ: ԱՌԱՋՆԱՅԻՆ ԿԱՌՈՒՑՎԱԾՔԸ ԵՎ ԴԵՐԸ ԱԶԴԱՆՇԱՆԻ ՏԵՂԱՓՈԽՄԱՆ ԵՎ ՀԱՐԹ ՄԿԱՆԻ ԿԾԿՈՂԱԿԱՆՈՒԹՅԱՆ ՄԵՋ

Ա.Ա.Գալոյան

Հոդվածում նկարագրված է մեր կողմից հիպոթալամուսից անջատված մի շարք նոր իմունոմոդուլյատորների քիմիական կառուցվածքը, այդ թվում իմունոսուպրեսոր FK-506-ի ռեցեպտոր – իմունոֆիլինի քիմիական կառուցվածքը: Վերջինս համարվում է նաեւ ֆերմենտ-պեպտիլ-պրոֆիլ-ցիս-տրանսիզոմերազ: Մեր կողմից հայտնաբերված մի շարք նեյրոպեպտիդներ խթանում են հակամարմինների առաջացումը կամ արգելակում այն:

Առաջ է քաշվում մի հիպոթեզ, ըստ որի հիպոթալամուսի էնդոկրին բջիջների մի մասը գլիալ բջիջների հետ միասին կատարում են T եւ B- լիմֆոցիտների դեր, արտադրում են իմտերլեյկիններ եւ հակամարմիններ,հատկապես նյարդային բջիջների ինֆեկցիոն ախտահարման ժամանակ:

ИММУНОМОДУЛЯТОРЫ ГИПОТАЛАМУСА. ПЕРВИЧНАЯ СТРУКТУРА И РОЛЬ В ПЕРЕДАЧЕ СИГНАЛОВ И СОКРАТИМОСТИ ГЛАДКОЙ МУСКУЛАТУРЫ

А.А.Галоян

Фундаментальная роль иммунокомпетентных Т-клеток, как и В-клеток, продуцирующих антитела против внешних антигенов в иммунной системе, хорошо известна.

Ряд исследований показывает потенциальную структурную связь между нервной и иммунной системами. Ряд нейропептидов избирательно модулирует гематологические и иммунологические свойства

лейкоцитов. Наши исследования показали способность нового класса гипоталамических нейропептидов модулировать антителообразование в индуктивном и продуктивном периодах иммунного ответа к поликлональным Т-зависимым (SRBC), Т-независимым (DNP-ficoll) антигенам in vitro и in vivo. Многочисленные наши исследования выявили наряду с кардиоактивными гормонами гипоталамуса также ряд иммуномодуляторов в мозге.

Накопленные нами литературные данные позволили выдвинуть гипотезу о том, что наряду с регулирующим воздействием нервной системы на иммунную мозг сам является иммунным органом и что гипоталамические нейросекреторные клетки вместе с глиальными клетками могут играть роль Т- и В-лимфоцитов, продуцирующих в конечном счете интерлейкины и ряд других иммуномодуляторов. Так, например, из нейросекреторных областей гипоталамуса мы изолировали ряд иммуномодуляторов, их первичная структура была идентифицирована (тимозин β_4, тимозин β_4 (1-39), тимозин β_1 (1-74, 1-76), фрагмент (43-51) и (72-85) основного белка миелина (FGSDRGPPK) и (EKAQGHRPQDENPY). Был идентифицирован также иммунофилин FK-506, связывающий белок. Этот белок обладает ферментативной активностью пентидил-пролин-цис-транс-изомеразы, играющий важную роль в механизмах биосинтеза не только интерлейкинов, но и катехоламинов (т.е. принимают участие в трансмиссии нервного импульса). Из состава гипоталамического порошка, полученного по методике А.А.Галояна совместно с Б.Гурвиц (1968), нам удалось выделить парвальбумин и расшифровать его первичную структуру. Удалось раскрыть структуру двух больших фрагментов: TDLLHAEDIKKAVGAFSAVD и VGLLKKXDIDVKKVF (х-неизвестная аминокислота). Это парвальбумин альфа (PRVA). Однако структура гипоталамического PRVA не полностью идентична с известными PRVA. Не имеются гомологии в аминокислотной последовательности в положениях N-7, T-21, K-36, K-38, A-40, D-41, K-45. По предварительным данным, ряд изолированных нами иммуномодуляторов, а также PRVA образуют комплекс с иммунофилином. Задача состоит в том, чтобы выделить иммунофилин и другие соединения из отдельных клеток гипоталамуса.

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