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Abstract

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SHIFTS IN CONTENT OF INTERLEUKINS IL-1 alpha, IL-2, IL-6 AND gamma-INTERFERON, PROLACTIN AND INSULIN-LIKE GROWTH FACTOR IN ORGANS OF IMMUNOGENESIS UNDER THE CONDITIONS OF PUTRESCINE ADMINISTRATION TO EXPERIMENTAL ANIMALS

Method of immune enzyme assay (ELISA) was employed to study the shifts in content of immunocytokines, prolactin, and insulin-like growth factor-1 in the central and peripheral organs of immunogenesis in mice. Single intravascular administration of putrescine was performed at rather low doses similar to levels determined in blood serum of small laboratory animals (rats and mice). Animals were euthanized by decapitation under the Nembutal narcosis in accordance with the strict requirements of Yerevan State Medical University (YSMU) Committee on Bioethics for investigations involving laboratory animals.

Shifts revealed in thymus of experimental animals signified putrescine-dependent immune-suppressive effect regarding the selective inhibition of IL-2, IL-6 and γ-IFN synthesis in the central organs of immunity. High indices of IL-1α, IL-6 and prolactin were recorded in the spleen and nodes of experimental animals 8 hours after the putrescine administration. It is not excluded that under conditions of our experiment, IL-6 high levels in spleen and lymph nodes were also conditioned by stimulant effect of prolactin towards B-lymphocytes populations (this effect is induced by relatively high levels of prolactin); besides that, similar mechanism of prolactin direct stimulating influence on B-lymphocytes in the aspect of IL-1α synthesis by B-lymphocytes is described in the literature.

It is also known that the enhanced synthesis of γ-IFN in immunocompetent cells is accompanied with the marked activation of specific T-lymphocytes sub-populations: T-suppressors and T-killers possessing the cytotoxic activity. Therefore, we might make an assumption: exogenous putrescine administered by us to the animal organism at rather low concentrations directly and/or indirectly inhibits the activity of cytotoxic T-lymphocytes sub-populations.

Key words: putrescine, organs of immunogenesis, B-lymphocytes, immunocytokines, prolactin, immunomodulation.

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Резюме

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ЗМІНИ ВМІСТУ ІНТЕРЛЕЙКІНІВ IL-1 α, IL-2, IL-6, γ-ІНТЕРФЕРОНУ, ПРОЛАКТИНУ ТА ІНСУЛІНОПОДІБНОГО ФАКТОРУ РОСТУ В ОРГАНАХ ІМУНОГЕНЕЗУ ПРИ ВВЕДЕННІ ПУТРЕСЦИНУ ЕКСПЕРИМЕНТАЛЬНИМ ТВАРИНАМ

Ми використали метод твердофазового імуноферментного аналізу (ELISA) для вивчення змін вмісту імуноцитокінів,

пролактину та інсуліноподібного фактору росту в центральних та периферичних органах імуногенезу мишей.

Було зроблено однократне внутрішньовенне введення путресцину в малій дозі, подібній до тієї, що була визначена в сиворотці крові лабораторних тварин (щурів та мишей). Під наркозом (препарат нембутал) тваринам робили декапітацію дотримуючись норм про дослідження з використанням лабораторних тварин, затверджених Єреванським державним медичним університетом.

Зміни, відмічені в тимусі експериментальних тварин, вказують на путресцин-залежний імунопригнічуючий ефект, який направлений на вибіркове уповільнення синтезу IL-2, IL-6 та γ-IFN в центральних органах імунної системи. Високі показники вмісту IL-1α, IL-6 та пролактину були визначені у селезінці та лімфатичних вузлах експериментальних тварин через 8 год після введення путресцину. Не виключено, що в умовах нашого експерименту високий рівень IL-6 в селезінці та лімфатичних вузлах був обумовлений стимулюючим ефектом на популяцію В-лімфоцитів (відносно високий вмістом пролактину викликав цей ефект). Крім цього, в літературі представлені дані про схожий механізм прямої стимуляції пролактином В-лімфоцитів, а саме синтез IL-1α В-лімфоцитами.

Відомо, що посилений синтез γ-IFN в імунокомпетентних клітинах супроводжується помітною активацією специфічних субпопуляцій Т-лімфоцитів: Т-супресорів та Т-кілерів, які мають цитотоксичну активність. Тому, ми можемо припустити, що путресцин, який ми ввели тваринам екзогенно в малих концентраціях, прямо та/чи опосередковано пригнічує цитотоксичну активність субпопуляцій Т-лімфоцитів.

Ключові слова: путресцин, органи імуногенезу, В-лімфоцити, імуноцитокіни, пролактин, імуномодуляція.

Резюме

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ИЗМЕНЕНИЯ СОДЕРЖАНИЯ ИНТЕРЛЕЙКИНОВ IL-1α, IL-2, IL-6, γ-ИНТЕРФЕРОНА, ПРОЛАКТИНА И ИНСУЛИНОПОДОБНОГО ФАКТОРА РОСТА В ОРГАНАХ ИММУНОГЕНЕЗА ПРИ ВВЕДЕНИИ ПУТРЕСЦИНА ЭКСПЕРИМЕНТАЛЬНЫМ ЖИВОТНЫМ

Мы использовали метод иммуноферментного твердофазового анализа (ELISA) для определения изменений содержания иммуноцитокинов, пролактина и инсулиноподобного фактора роста в центральных и периферических органах иммуногенеза мышей.

Мы однократно внутривенно вводили путресцин в малой дозе, подобной к той, что была определена в сыворотке крови лабораторных животных (крыс и мышей). Под наркозом (препарат нембутал) животных декапитировали. Эту процедуру проводили следуя нормам об исследованиях лабораторных животных, которые были утверждены Комитетом по биоэтике Ереванского государственного медицинского университета.

Изменения, замеченные в тимусе экспериментальных животных, указывают на путресцин-зависимый иммуноингибирующий эффект направленный на выборочное угнетение синтеза IL-2, IL-6 та γ-IFN в центральных органах иммунной системы. Высокие показатели

содержания IL-1α, IL-6 и пролактина были отмечены в селезенке и лимфатических узлах экспериментальных животных через 8 ч после введения путресцина. Не исключено, что в условиях нашего эксперимента, высокий уровень IL-6 в селезенке и лимфатических узлах был обусловлен стимулирующим эффектом, направленным на популяцию В-лимфоцитов (относительно высокий уровень пролактина вызвал этот эффект). Кроме этого, в литературе представлены данные о похожем механизме прямой стимуляции В-лимфоцитов пролактином, а именно синтез IL-1α В-лимфоцитами.

Известно, что усиленный синтез γ-IFN в иммунокмпетентных клетках сопровождается заметной активацией специфических субпопуляций Т-лимфоцитов: Т-супрессоров и Т-хелперов, обладающих цитотоксической активностью. Поэтому, мы можем предположить, что путрсцин, введенный нами в малой концентрации, прямо и/или опосредованно угнетает цитотоксическую активность субпопуляций Т-лимфоцитов.

Ключевые слова: путресцин, органы иммуногенеза, В-лимфоциты, иммуноцитокины, пролактин, иммуномодуляция.

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Introduction

Recent studies revealed that cytokines have direct and indirect influence on processes of synthesis and transport of biogenic polyamines [5, 6]. Thus, it was established that IL-1α and 1β, TNF-α in spleen, liver, and bone marrow of mice stimulate the activity of ornithine decarboxylase (ODS), which leads to the increase of putrescine content in these organs. In addition, same authors showed that IL-2, IL-6, and γ-interferon (γ-IFN) produce effects to synthesize putrescine in the same organs. To our mind, this latter signifies the selective stimulating effect of specific immunocytokines, which activate ornithine decarboxylase in parenchymatous organs with subsequent synthesis of putrescine. There is evidence that accumulation of putrescine in a number of extreme states in the heart is accompanied by remodeling processes in parenchymatous and stromal elements of myocardium. Moreover, the structural rearrangement of myocardium occurs either by the way of hematogenic or lymphogenic entry of cytokines to the myocardium or as a result of their synthesis (*in situ*) [13, 14].

In the mentioned aspect, one should emphasize that mediatory link of the immunity was not the subject of a special investigation associated with the character and peculiar features of putrescine synthesis in internal organs under conditions of normally functioning organism [15]. Meanwhile, it was found that numerous effects of putrescine are directly or indirectly mediated precisely by its low concentrations, which are practically similar to concentrations of many endogenously active factors of hormonal activity spectrum. Therefore, in our opinion, investigation aimed at studies on biological role of putrescine in processes of immunocompetent cells modulation – in the aspect of selective synthesis and/or inhibition of immunogenesis in organs of immune genesis, – seems quite promising [14].

Material and Methods

The study was performed on 48 white male mice weighing 35–40 g. The control group included intact animals. Animals of experimental series were divided into 2 groups. Animals of experimental groups 1 and 2 were withdrawn from the experiment 2 hours (h) and 8 h, respectively, after the single intravascular administration of putrescine (Sigma, USA) given as 10^{-9} mg/mL per 100 g animal body weight. Animals of the control group were exposed to intravascular administration of 0.1 mL saline, i.e. such an amount of saline that was used to dissolve putrescine for administration to animals of experimental groups. Prior to euthanasia animals were exposed to Nembutal narcosis under observance of all standards of bioethical control set forth by the YSMU Committee on Bioethics for experimental investigations.

IL-1, IL-2, IL-6 and γ-IGF levels in the heart were determined using the immune enzyme assay method (ELISA) and the corresponding kits of

"DRG International Inc." (USA - Germany). Prolactin and IGF-1 levels in the heart were determined using Mouse/Rat Prolactin ELISA, GenWay Biotech, Inc. and IGF-1 (Mouse, Rat) ELISA ALPCO Diagnostic. IL-1, IL-2, IL-6 and γ-IGF contents were expressed in pg/mL, while for prolactin and IGF-1 the measurement ng/mL was used.

Blood was collected from laboratory mice after decapitation. The blood was stored at room temperature (20ºC) for 2 h and then centrifuged at 1500 xg at 4°C for 20 min. The serum was separated and stored at –20°C until being assayed for melatonin.

Immediately after the blood collection, the organs of immunogenesis (thymus, spleen, mesenteric lymph nodes) were rapidly removed, weighed and homogenized in 0.4 mol/L $HClO_4$ (4 ml/g tissue) at 0–4°C, using a teflon glass cell disrupter at the maximal speed (1000 rpm). After 15 min at 0–4°C, homogenates were centrifuged at 16000 xg for 20 min, and the supernatants frozen at –28°C until the immune enzyme assay.

A summary count of the immune enzyme assay results was done on the "Stat Fax 3200" automatic analyzer (Awareness Technology Inc., USA) according to the requirements of the Instructions on determination methods for each kit. Plotting the calibration curve and determination of samples concentrations were done by means of Delta Graf program (Version 5.4).

All statistical analyses were performed using SPSS version 13.0 software package for Microsoft Windows (SPSS, Inc., Chicago, IL). Data are presented as mean ±SEM. Comparisons between groups were performed using Student's t test and ANOVA.

Results

According to results of the immune enzyme assay, putrescine administration to mice was accompanied by multidirectional shifts of immunocytokines content in the central and peripheral organs of immunity (Table 1).

It should be noted that the content of interleukins-Iα, -2, -6 and γ-interferon in thymus of experimental animals; namely: indices of interleukins-Iα, -2, -6 and γ-interferon 2 hours post putrescine administration were lower 3.4; 7.4; 2.6 and 2.7-fold, respectively. Eight hours after the putrescine administration a marked decrease in interleukins-2, -6 and γ-interferon levels also occurred in thymus and the indices were lower than in the controls 24.7; 2.5 and 1.6-fold, respectively. The level of interleukin-Iα was normalized in the mentioned period of observation. Somewhat different picture was observed in spleen and lymph nodes of experimental animals. In particular, 2 hours after the putrescine administration low levels of interleukins-2 and γ-interferon were registered in spleen: 3.8 and 1.9 times lower compared with the controls. Indices of Il-1α and Il-6 were within the range of control values. Eight hours after the putrescine administration, indices of interleukin-2 came to norm in both peripheral organs of immunity and in thymus; however, against this background further decrease of γ-interferon took place, the level of which was below the control level 2.5 and 2.2-fold, respectively, in the spleen and lymph nodes of experimental animals. One should emphasize the fact that 8 hours after the putrescine administration the level of Il-1α and Il-6 significantly increased in spleen and lymph nodes. Thus, the level of interleukin-1α in spleen and lymph nodes was above the control level, exceeding it 3 and 3.5-fold, while the level of interleukin 6 was higher 1.9 and 2.2-fold, respectively. It is noteworthy that high levels of Il-1α and Il-6 correlated with blood serum levels of mentioned cytokines. Eight hours after putrescine administration to the organism of experimental animals there was a marked increase of interleukins Il-1α and Il-6 in blood serum. These indices exceeded the controls 6.3 and 1.7-fold, respectively. Two hours after the putrescine administration no significant shifts of IL-Iα, IL-2 and IL-6 were observed. In this period of observation, mentioned indices were within the range of control values; likewise, in blood serum, and immunity organs, a marked decrease of γ-interferon levels took place. The level of γ-interferon after the putrescine administration was 2.2 times below the control level; in 8 hours, it was 3 times lower.

We consider expedient to interpret the revealed changes in cytokines content from the point of view of neuroendocrine shifts conditioned by prolactin and insulin-like growth factor, as these hormones are currently considered as immunomodulators of targeted spectrum of action in the aspect of stimulation and/or inhibition of specific pro-inflammatory cytokines.

The results of performed immune enzyme assay are presented in Table 2. As is evident from Table 2, 2 hours after the putrescine administration the level of prolactin in the central and peripheral

organs of immunity does not actually differ from the control level.

However, 8 hours after the putrescine administration the level of prolactin in thymus, spleen and lymph nodes markedly increased and exceeded control indices 1.6, 2.5 and 1.75-fold. In the mentioned period of observation we recorded relatively high blood serum indices of the indicated growth hormone. Namely: blood serum level of prolactin in experimental animals was 1.9 times above the control level 8 hours after the putrescine administration. The immune enzyme assay performed for determination of insulin-like growth factor in the organs of immunity did not reveal any significant shifts in the content of the mentioned hormone: indices of IGF-1 were within the range of control values in all time intervals of observation. However, we recorded relatively high indices of insulin-like growth factor-1 in the blood serum of experimental animals 8 hours after the putrescine administration, the level of which 2 times exceeded the control level.

Table 1

Shifts in content of interleukins 1, 2, 6 and γ-interferon in blood serum and organs of immunogenesis under conditions of putrescine administration to experimental animals

Object of study	Study groups	Studied indices			
		IL-1α	IL-2	IL-6	γ-IGF
Blood serum	Control	25.4 ± 5.1	232.4 ± 56.8	1661.5 ± 332.2	12.5 ± 2.3
	Experimental group 1 (in 2 hours)	32.6 ± 6.5 0.25 > p > 0.1	393.2 ± 78.6 0.1 > p > 0.05	1196.4 ± 239.2 0.25 > p > 0.1	5.7 ± 1.4 0.025 > p > 0.01
	Experimental group 2 (in 8 hours)	159.6 ± 31.9 p = 0.0005	109.1 ± 28.6 0.05 > p > 0.025	2893.6 ± 306.7 0.01 > p > 0.005	4.2 ± 1.5 0.005 > p > 0.0005
Thymus	Control	131.2 ± 26.2	654.3 ± 1308.6	1013.3 ± 202.6	16.2 ± 3.2
	Experimental group 1 (in 2 hours)	38.2 ± 7.6 0.005 > p > 0.0005	886.8 ± 177.4 p < 0.0005	384.6 ± 56.9 p = 0.005	6.3 ± 1.8 0.01 > p > 0.005
	Experimental group 2 (in 8 hours)	163.7 ± 32.7 0.25 > p > 0.1	264.2 ± 52.8 p < 0.0005	408.3 ± 63.1 0.01 > p > 0.005	10.0 ± 1.4 0.05 > p > 0.025
Spleen	Control	83.9 ± 16.8	682.8 ± 136.6	1136.2 ± 182.5	4.9 ± 0.4
	Experimental group 1 (in 2 hours)	80.3 ± 13.5 p > 0.4	177.4 ± 25.4 0.005 > p > 0.0005	1397.8 ± 165.4 0.25 > p > 0.1	2.6 ± 0.5 0.005 > p > 0.0005
	Experimental group 2 (in 8 hours)	252.7 ± 40.5 0.005 > p > 0.0005	886.3 ± 169.5 0.25 > p > 0.1	2185.6 ± 226.9 0.005 > p > 0.0005	1.9 ± 0.7 0.005 > p > 0.0005
Lymph nodes	Control	69.7 ± 16.3	594.5 ± 90.5	1170.8 ± 120.0	5.9 ± 0.8
	Experimental group 1 (in 2 hours)	90.6 ± 20.8 0.25 > p > 0.1	238.1 ± 38.6 0.005 > p > 0.0005	1339.9 ± 149.3 0.25 > p > 0.1	2.4 ± 0.5 0.005 > p > 0.0005
	Experimental group 2 (in 8 hours)	241.6 ± 50.8 0.005 > p > 0.0005	487.6 ± 63.3 0.25 > p > 0.1	2544.8 ± 234.6 P < 0.0005	2.6 ± 0.6 0.005 > p > 0.0005

Table 2

Shifts in content of prolactin and insulin-like growth factor in blood serum, heart and organs of immunity under the conditions of putrescine administration to experimental animals

Object of study	Study groups	Studied indices	
		PRL	IGF-1
Blood serum	Control	5.3 ± 1.1	0.91 ± 0.06
	Experimental group 1 (in 2 hours)	4.3 ± 0.8 0.25 > p > 0.1	0.8 ± 0.07 0.1 > p > 0.05
	Experimental group 2 (in 8 hours)	10.2 ± 2.1 0.05 > p > 0.025	1.85 ± 0.3 0.005 < p > 0.0005
Thymus	Control	1.7 ± 0.2	0.61 ± 0.05
	Experimental group 1 (in 2 hours)	1.8 ± 0.2 0.4 > p > 0.25	0.93 ± 0.2 0.1 > p > 0.05
	Experimental group 2 (in 8 hours)	2.7 ± 0.5 0.1 > p > 0.05	0.84 ± 0.3 0.25 > p > 0.1
Spleen	Control	2.4 ± 0.6	0.73 ± 0.04
	Experimental group 1 (in 2 hours)	2.8 ± 0.5 0.4 > p > 0.25	0.78 ± 0.05 0.025 > p > 0.1
	Experimental group 2 (in 8 hours)	6.0 ± 1.2 0.025 > p > 0.01	0.64 ± 0.09 0.25 > p > 0.1
Lymph nodes	Control	2.0 ± 0.1	0.47 ± 0.03
	Experimental group 1 (in 2 hours)	2.5 ± 0.3 0.1 > p > 0.051	0.57 ± 0.1 0.25>p>0.1
	Experimental group 2 (in 8 hours)	3.5 ± 0.6 0.025 > p > 0.01	0.43±0.04 0.25 > p > 0.1

Discussion

Our findings indicate that single administration of putrescine at rather low concentrations to intact animals was accompanied by differently directed shifts in the content of studied cytokines in thymus, spleen, lymph nodes, and blood serum. Thus, the revealed shifts in thymus apparently signify putrescine-dependent immune-suppressive effect as regards the selective inhibition of specific cytokines synthesis in the central organs of immunity. It is not ruled out that such a mechanism was conditioned by exogenous putrescine that was administered to experimental animals.

In available literature, there is evidence on the influence of polyamines and products of their oxidation towards IL-2 secretion processes by immunocompetent cells.

In this respect, it is noteworthy to mention the research performed by E. Flescher and co-authors since 1988 to 1994.

In particular, E. Flescher and co-authors (1989) established that the biosynthesis of polyamines by mononuclear cells regulates the activity of T-lymphocytes populations. Particularly it was shown that the products of polyamine oxidase and spermidine interaction have an inhibiting influence on IL-2 production in immunocompetent cells. In this aspect, the authors expressed a rather pivotal assumption, according to which there are “loops” functioning on the principles of feedback in processes of polyamines and IL-2 synthesis. Analogous effect was obtained by the authors [8] upon studies of similar correlation in patients with rheumatoid arthritis (RA). In particular, the authors revealed that IL-2 production by mononuclear cells of RA patients’ synovial membrane was inversely proportional to concentrations of polyamines in mentioned cells.

The obtained results were supported by special investigations, in which ornithine decarboxylase

(ODC) activity was blocked due to addition of difluoromethylornithine (DFMO) lymphocytes, as ODC activity specific blocker (to culture medium), as a result of which interleukin-2 synthesis by immunocompetent cells was enhanced [7, 9]. The same authors established the molecular mechanisms of this phenomenon as well [10]. Due to studies performed by other authors [2–4], similar results were obtained regarding the inhibitory potencies of polyamines towards the proliferative activity of lymphocytes.

Upon analysis of literature data on inhibitory influence of polyamines and products of their oxidation on processes of interleukin 2 secretion the following fact seems slightly paradoxical at the first glance: against the background of a significant decrease of IL-Iα, IL-2, IL-6 and γ-interferon in the central organs of immunity, rather high indices of IL-Iα were recorded in spleen and lymph nodes 8 hours after the putrescine administration to the organism.

Taking into consideration that a high level of IL-Iα in spleen and lymph nodes was recorded in our studies at a relatively delayed period of the experiment, i.e. 8 hours after putrescine administration, we can assume that in IL-Iα production stimulating processes in peripheral organs of immunogenesis putrescine-dependent hormonal mechanisms are also involved.

First of all, prolactin is meant. According to the literature data [1, 12, 11], prolactin activates specifically B-lymphocyte population in spleen and lymph nodes in a dose-dependent manner, thus stimulating the processes of antibody formation. As shown above, in our experiment the level of prolactin significantly increased in both peripheral organs of immunity 8 hours after the putrescine administration. Supposition might be drawn that 8 hours after putrescine administration against the background of high prolactin concentrations in both blood serum and peripheral organs of immunity a structural rearrangement of lymphoid tissue accompanied by activation of humoral immunity reactions occurs. Although it is generally recognized that macrophages and B-lymphocytes population serves as a main source of IL-Iα synthesis in the mammalian organism within the framework of the immune system functioning. Therefore, in our opinion, the mechanism of mediated (prolactin-dependent) activation of B-lymphocytes population of spleen and lymph nodes, which is selectively directed towards both activation of humoral immunity reactions and IL-Iα production by B-lymphocytes, seems quite realistic.

Hence, we cannot exclude that amongst the immunocompetent cells of spleen and lymph nodes, B-lymphocytes populations, the content of which significantly increased in specific structures under administration of putrescine, serve as a source of enhanced IL-6 synthesis.

Conclusion

In our opinion, low level of γ-IFN observed in the central and peripheral organs of immunogenesis throughout the experiment signify immune suppressive action of putrescine on T-lymphocytes sub-populations. It is known that the enhanced synthesis of γ-IFN in immunocompetent cells is accompanied with the marked activation of specific T-lymphocytes sub-populations: T-suppressors and T-killers having cytotoxic activity. Therefore, an assumption might be made according to which exogenous putrescine, administered to the animal organism in rather low concentrations directly and/or indirectly, inhibits the activity of cytotoxic T-lymphocytes subpopulations, – in the aspect of γ-IFN selective synthesis by them.

We cannot exclude that in the organism of mammals earlier unknown putrescine-dependent functional loops of immune processes modulation are engaged, in mechanisms of which the significant role belongs to prolactin as well.

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