Genetically determined mechanisms of arterial hypertension related to dietary calcium deficiency (parathyroid hypertensive factor)

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Abstract

The paradigm of the parathyroid hypertensive factor (PHF) and its role in the development of systemic hypertension (HTN) in relation to dietary calcium deficiency was analyzed on the basis of available epidemiological, clinical and physiological data. Modern conceptions of molecular and cellular mechanisms of HTN were considered. Molecular-genetic mechanisms of HTN in humans and in animals are similar. The PHF presence was found in blood plasma in hypertensive patients, as well as in blood of spontaneously hypertensive rats (SHR) kept on low calcium diet. The experimental results show that under the conditions of low Ca^{2+} intake the blood pressure increases only six weeks after birth preceded by the elevation of Ca^{2+} level in vascular smooth muscle cells and cardiomyocytes, as well as metabolic changes in neuroregulatory proteins in the central nervous system. The NAP-22 fraction of neurospecific brain peptides appears to be an informative biomarker of calcium dependent HTN. With the use of polymerase chain reaction with reverse transcription (considering the presence of PHF in human blood), a reliable method is proposed to identify the genes encoding PHF-dependent HTN. This method would help to detect the proneness to the disease, and will enable and early primary HTN prevention in certain population groups.

Key words: hypertension, parathyroid hypertensive factor, low calcium intake, biomarkers, NAP-22.

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Генетически детерминированные механизмы развития артериальной гипертензии при дефиците экзогенного кальция (паратиреоидный гипертензивный фактор)

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Резюме

На основе анализа современных эпидемиологических, клинических, физиологических данных, а также представлений о молекулярных и клеточных механизмах формирования артериальной гипертензии (АГ) дана характеристика формирующейся в настоящее время парадигмы о роли паратиреоидного гипертензивного фактора (ПГФ) в развитии некоторых форм АГ при дефиците экзогенного кальция. Молекулярно-генетические механизмы формирования АГ у человека и животных сходны. Продемонстрировано присутствие ПГФ в крови некоторых больных АГ, а также в эксперименте у спонтанно гипертензивных крыс линии SHR (экспериментальная модель АГ у человека), содержавшихся в условиях дефицита поступающего в организм Ca^{2+}. Результаты, полученные в эксперименте, свидетельствуют о том, что в условиях дефицита поступающего в организм Ca^{2+} повышение артериального давления формируется в онтогенезе не сразу, а лишь через 6 недель после рождения (еще в предгипертензивную фазу), на фоне генетически детерминированных нарушений обмена внутриклеточного Ca^{2+}, нарастающей концентрации Ca^{2+} в гладкомышечных клетках сосудов и кардиомиоцитах, а также изменений в метаболизме нейрорегуляторных белков в центральной нервной системе. Фракция NAP-22 нейроспецифических пептидов мозга является информативным биомаркером кальцийзависимой АГ. С использованием широко доступного метода полимеразной цепной реакции с обратной транскрипцией (учитывая присутствие в крови человека ПГФ) предполагается получить надежный метод выявления в крови человека генов, кодирующих развитие ПГФ-зависимой АГ. Метод позволит, с одной стороны, выявить лиц, предрасположенных к развитию патологии, а с другой — вплотную подойти к направленной широкой первичной профилактике АГ в некоторых группах населения.

Ключевые слова: артериальная гипертензия, паратиреоидный гипертензивный фактор, дефицит экзогенного кальция, биомаркеры, NAP-22.

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Calcium is the chemical element that plays main role in biologic systems of living bodies and controls the majority of key processes inside cell. It can execute either static function in stable structures or dynamic function by taking part in transmitting signal as second cell messenger.

Nowadays pathogenesis of illnesses related to calcium metabolic disturbance is actively studied all over the world. Calcium metabolism disorders were found in some forms of hypertension (HTN). Underlying mechanisms are genetic disorders of calcium metabolism providing constant intracellular level of free unbound calcium [Ca2+]. It contains in vascular smooth muscle cells (SMC), cardiomyocytes (CMC), and brain neurons.

Intracellular calcium concentration differs from extracellular level in several orders. Thus, there exists a complicated system to control [Ca2+] level. It consists of channels, exchangers and pumps of plasmolemma and intracellular membranes. There is a lack of data about activation of signaling cascade that uses [Ca2+] as a second messenger in HTN and genetic metabolic disturbances.

Epidemiological [1–4] and clinical [5–13] trials, and experiments on animals [9, 14–17] as well molecular studies of intracellular signaling pathways have shown a correlation among nutritional calcium level and HTN development [18]. The results evidence the existence of a threshold of calcium consumption (400–500 mg/day). Lower level is associated with the HTN risk increase in several times. Calcium containing in drinking water in bioavailable form of free ions has a high physiological significance [3,7–9, 14–17, 19–23]. In particular, calcium deficiency in drinking water is one of the factors modifying membrane iontransporting system in cells (Na+, K+, Cl– — cotransport). These are genetically linked to the development of sodium sensitivity and to volume-dependent HTN [24–26]. Thus, an increase of sodium consumption for

100 mmol leads to an increase of calcium excretion for 1 mmol. The reduction of sodium consumption from 200 to 50 mmol/day is associated with the decrease of daily loss of calcium for 45% (from 4,8 to 3,4 mmol).

In addition, experimental and clinical results evidence that calcium impedes the development of salt-induced HTN (experiments on animals and among the patients). Moreover, restoring of calcium level leads to a decrease of salt-sensitivity [9, 11, 25–31].

Series of studies demonstrated an antihypertensive response of consumed calcium in patients AHTN. Meanwhile, experiment on spontaneously hypertensive rats (SHR, an experimental model of primary hypertension in humans) showed that calcium rich food prevents HTN progression and stress-induced elevation of blood pressure (BP).

The conception of dietary calcium influence on vascular tone and HTN progression was formed long ago. In spite of this, it was approved by scientific society only in the end of last century after the discovery of a new hypertensive factor. So-called parathyroid hypertensive factor (PHF) was discovered by a group of Canadian scientists in 1987 [28–46]. Today they are leaders in PHF research [47] (more than 11 international projects had been held by 1992). PHF sharply differs in its chemical structure and physiological properties from all formerly known calcium-regulating hormones, including the parathyroid hormone.

PHF was found in blood plasma in SHR rats and in many patients with HTN. After the injection of PHF-containing blood plasma to normotensive animals their BP raised significantly [14–17, 20,44]. Also there was a rapid decrease of BP to normal level after stopping the injection of SHR blood plasma and replacing it with plasma of normotensive animals. It acts in the same way in humans [28, 42].

It is well-known that calcium channel blockers inhibit PHF activity [27, 42]. Injection of nifedipine prevents from BP increase after the bolus dosing of PHF in vivo. It increases L-type calcium channels in vascular SMC and CMC activity, potentiates noradrenaline effects on vascular wall. Also it causes the concentration growth of intracellular Ca2+ mediated by KCl, associated with the cell membrane depolarization and opening of the voltage-gated calcium channels [27, 42]. PHF was shown to suppress the voltage-gated potassium channels activity in vascular SMC membranes [41, 42], and also to change the characteristics of tetrodotoxin-sensitive sodium channels [48].

Thus, PHF indirectly regulates the Ca2+ entrance into cell and at the same time get inhibited in case of adequate calcium intake. PHF molecular weight is 2700D. In experiments it causes delayed BP rise in normotensive animals, coinciding with the increased flow of extracellular Ca2+ in vascular SMC [42].

PHF active component consists of the oligopeptide connected with the phosphoglyceride. It also causes a delayed BP increase in normotensive animals. This effect coincides with the increased inflow of extracellular calcium inside the cell. The antibodies to the PHF active component were obtained and are used in immunoenzymometric assay [44, 45] to assess the presence of this factor in other diseases associated with the increased calcium inflow in cells. PHF is used as a diagnostic marker of salt-sensitivity and low-renin HTN, and is considered as the reason of HTN development [27, 28]. PHF-positive (salt-sensitive) hypertensive patients were shown to respond to calcium channel antagonists and diuretics therapy meanwhile PHF-negative patients have higher response to angiotensin converting enzyme inhibitors and beta-blockers [42–46]. PHF antagonists also can be used for the management of other diseases associated with the increased intracellular calcium concentration [42].

There usually are 5–20 amino-acid residues (more often 5–10) in the polypeptide of PHF active component. In general its structure has next sequence Tyr-Ser-Val-Ser-His-Phe-Arg. According to a mass-spectrometry PHF molecular weight is 1000–2700D and it can be increased by adding other polypeptides.

PHF active component can be connected with biologically active substance or any molecule (hormones, diuretics, neurotransmitters) that could serve as a PHF transporter to the specific binding sites in cells, tissues and organs.

Biologically active substances may have high affinity to different cells including SMC and CMC. Ways of fixation of these substances with the PHF active component are well-known [42].

In cooperation with the Institute of Macromolecular Compounds of Russian Academy of Sciences and the Institute of Highly Pure Biopreparations we gained a structural and functional synthetic PHF analogue that has full range of its physiological effects. The antibodies to this compound were produced. The fraction with the highest dilution to PHF later on was used in the immune-enzyme assay. As a result of immunization of animals (rabbits) a polyclonal antiserum with an evident antihypertensive effect was obtained [46, 49].

By present moment there are no available and reliable methods of identification of the gene or genes encoding the amino-acid PHF sequence.

Apparently, PHF maybe considered as one of the main reasons in the development of calcium-dependent HTN, especially wide-spreaded in the regions with low microelement concentration in natural drinking water [3, 7, 8], and of some other cardiovascular diseases [50]. That is why the search of a quantitative and available method of PHF detection in blood is quite actual nowadays.

The intensive development of new molecular technologies in biology and medicine opened new ways for biomarker search. Their presence in blood is associated with the circulating PHF. These biomarkers provide the diagnosis of illnesses, even at early stages. Comparing the results of mass-spectrometry and immune-enzyme assay may become a perspective way for developing a new diagnostic tool for identification of calcium-dependent HTN and other illnesses caused by nutritional calcium deficiency.

Today it is hard to find any other issues in medicine that could develop so fast and bring such new and important information as the problem of genetic and environmental relations and their role in disease progression. The understanding of molecular mechanisms underlying the vascular tone increase in HTN, myocardial disorders and structural and functional changes of other target organs (brain, kidneys) is essential to solve out the fundamental problem of the development of calcium-dependent HTN and associated illnesses. Primary prevention and patient-specific HTN treatment are based on this approach [51] implying the detection of genetic factors and predictable response to the chosen medication. The most valuable result is expected from new clinical researches with regard to this hypothesis [51].

Rats with genetically determined spontaneous HTN along with elevated BP are also characterized by the noticeable changes in their behavior and cognitive processes, which significantly increase with aging [52, 53].

Molecular mechanisms of HTN progression are shown to be universal and similar in humans and rats. Experimental models provide the unique opportunity to study the consecutive activation of the cellular and molecular mechanisms of HTN progression in postnatal ontogenesis and give enough data for consistent conclusions. In particular, the SHR rats were shown to have impaired quantitative distribution and structural characteristics of proteins. Those proteins play an important role in integration processes in brain, take part in synaptic signaling and are reliable for memory formation and study processes [52, 53].

It is known that in SHR rats BP increases during early postnatal ontogenesis in 6–8 weeks after birth. Meanwhile, the genetic disorders of [Ca2+]i metabolism in vascular SMC and CMC are tracked since the first days after birth, right before the stable BP elevation, during the prehypertensive phase [18, 52, 53].

Neuroregulatory proteins of nerve-endings GAP-43 and NAP-22 (major substrates of protein kinase C) are some kind of markers of plastic processes in nervous system. They take part in study processes, cognitive functions through neuronal net formation, and axon growth during early postnatal ontogenesis [57, 58]. Therefore, along with circulatory dynamics indices we studied the mRNA expression, posttranslational and structural protein modifications in hypertensive and normotensive rats. Notable differences were found between the breeds exposed to the exogenous calcium deficiency and the rats supplied by fodder and water with normal calcium level. So, in SHR rats even in the first days after birth, i. e. long before the HTN development, there was a NAP-22 uptake in parietal cortex and of GAP-43 proteolysis enhancement in the synaptosomes [54–56].

Thus, the brain peptide set in these groups of animals differed by only one fraction — NAP-22 [54–56]. As its expression level in brain neurons in hypertensive SHR rats is much higher

than in normotensive rats it is suggested that NAP-22 may correlate with PHF presence in blood. The discovery of the markers in brain structures of Ca2+-deficient rats contributed further search of similar biomarkers in other biologic fluids and tissues, including those available for clinical investigation.

The Reverse Transcription PCR method (in case of PHF presence) might contribute to the development of a reliable method for genes identification in human blood that encode PHF-dependent HTN progression [59].

The studies dedicated to the influence of dietary calcium on BP level hav been carried out since 1983. The positive affect was found only in regions with a low calcium consumption. At the same time in regions with the normal rate of calcium consumption the impact was weak or lacking. That means that dietary calcium does not directly influence BP level, but its effect is mediated by calcium deficiency mechanisms.

On the one hand, the molecular and genetic approach to the pathogenesis of calcium-dependent HTN will enable finding of high risk groups of patients. On the other hand, it will bring closer the targeted correction of environment component and wide primary HTN prevention in some population groups. Apparently, diagnostics and prevention of such diseases is of great importance for the regions where the nutritional calcium deficiency increases due to low Ca2+ concentration in the drinking water, contributing to high prevalence of intracellular calcium exchange disorders (calcium-dependent HTN including HTN during pregnancy, osteoporosis, attention-defict/hyperactivity disorder in children, diabetes).

Thus, different pathologies related to the lack of calcium in food and water may have common genetic causes but differ in molecular mechanisms and development.

Conflict of interest. Authors declare no conflicts of interest.

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