Expression of NAP 22 mRNA in kidney of spontaneously hypertensive rats (SHR line) and normotensive rats (WKY line) in early postnatal ontogenesis under normal exogenous calcium intake and its deficit

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Abstract

In kidney of spontaneously hypertensive rats in early postnatal ontogenesis, we studied the changes in the expression of NAP-22 quantitatively predominant protein kinase C substrate mRNA, which served us as an indicator of the severity of disruption in cell apparatus. Exogenous calcium deficiency increased the expression of NAP-22 mRNA. Based on the changes in the expression level of this protein, it can be resumed that disorders of kidney function precede the formation of lasting hypertension in the animals.

Key words: spontaneously hypertensive rats (SHR), kidney, early postnatal ontogenesis, protein NAP-22, calcium deficiency.

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Экспрессия мРНК NAP-22 в почках крыс со спонтанной гипертензией (линия SHR) и нормотензивных крыс (линия WKY) в раннем постнатальном онтогенезе в условиях нормального поступления экзогенного кальция и его дефицита

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Резюме

Цель исследования. Изучить изменения уровня экспрессии мРНК NAP-22 в почках крыс со спонтанной гипертензией в различные периоды раннего постнатального онтогенеза и оценить их зависимость от уровня поступления в организм экзогенного кальция. **Материалы и методы.** В работе использовали крыс линий SHR и WKY в возрасте 5, 13, 18 и 30 дней. Уровень мРНК NAP-22 определяли в корковом и мозговом слоях почек методом полимеразной цепной реакции в реальном времени. **Результаты.** В период, предшествующий формированию стойкой артериальной гипертензии, у крыс линии SHR при достаточном поступлении кальция в организм уровень экспрессии мРНК NAP-22 в почках был ниже, чем у нормотензивных крыс. При дефиците поступающего кальция у крыс со спонтанной гипертензией уровень экспрессии мРНК NAP-22 существенно повышался по сравнению с нормотензивными WKY, у которых уровень экспрессии мРНК NAP-22 значимо снижался. **Выводы.** При дефиците экзогенного кальция у спонтанно гипертензивных крыс нарушается работа клеточного аппарата коркового слоя почек, о чем можно судить по компенсаторному увеличению уровня экспрессии мРНК NAP-22. Увеличение экспрессии мРНК NAP-22 у крыс SHR более выраженное, чем у WKY, можно связать с активацией внутриклеточных кальций-зависимых каскадов еще задолго до устойчивого повышения артериального давления.

Ключевые слова: спонтанная гипертензия, почки, дефицит экзогенного кальция, белок NAP-22, крысы линии SHR, онтогенез.

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Introduction

Pathogenesis of hypertension (HTN) and related diseases in exogenous calcium deficiency seems an unsolvable problem if there is lack of understanding of the molecular mechanisms of Ca2+ homeostasis inside target organ cells (myocardium, kidneys and brain). Previously, we have shown [1] an increase in the level of NAP-22 protein (in aggregated and unaggregated forms) in neurons of telencephalon at early stages of ontogenesis in spontaneously hypertensive rats with genetically determined disorders of intracellular calcium homeostasis.

NAP-22 is an universal regulator protein, one of the main targets of protein kinase C that is a calcium-dependent enzyme, responsible for phosphorylation of proteins, involved in transmission of cellular signals. In particular, NAP-22 is found in kidneys, and kidney functional disorders can change its metabolism [2]. In this regard, studying kidney NAP-22 at early stages of ontogenesis in spontaneously hypertensive rats might contribute to the better understanding of HTN pathogenesis and the role of exogenous calcium.

Whether the leading mechanism of HTN is the violation of glomerular perfusion, or intracellular processes in podocytes and glomerular smooth muscle cells (SMC) of the renal arteries, is not clear. To answer this question, the level of mitochondrial ribonucleic acid (mRNA) encoding NAP-22 protein was assessed in the cortical and medullar kidney layers in early postnatal ontogenesis (in age 5, 13, 18, 30 days before the development of resistant HTN).

NAP-22 protein is associated with the cytoskeleton [2], located in nucleus in kidney tissues; its cellular localization and expression may depend on the cell type [3]. Wilms-tumor suppressor protein WT1 frequently mutates in childhood renal tumors [4]; it can interact with protein NAP-22, which in this case functions as a transcriptional WTI co-suppressor [3]. Both proteins are found in podocytes, and level of mRNA NAP-22 expression may serve an indicator of pathological changes in kidneys, including those, associated with vascular tone [1].

Calcium intake plays an important role in spontaneous HTN formation in SHR rats [6]. Therefore, we evaluated expression dynamics

of mRNA NAP-22 both in normal and reduced calcium consumption.

Design and methods

The study was conducted on SHR rats and normotensive rats (WKY) of 5, 13, 18 and 30 days old of both genders. Animals were divided into two groups. Three generations of the animals form the first group received water with normal Ca level (80 mg/l) (recommendations of the World Health Organization). The second group received low-Ca-mineralized water (8 mg/l). Solid food contained daily dose of calcium in both groups.

The expression of mRNA NAP-22 was measured in the cortical and medullar kidney layers in both rat lines. Animals were kept in one cage with mother with free access to food and water. Twelve hour photoperiod was set.

In the third generation in both groups 5 rats at 5, 13, 18 and 30 days were taken for studies. The study was performed according to Helsinki Declaration (2008).

Kidney fragments were used for the extraction of total mRNA (Quick-RNA ™ MiniPrep Kit, Zymo Research). Reverse transcription was carried out using «Reverte» reagents. Real-time polymerase chain reaction (PCR) and termostating were performed using amplificator ANC-32 (Institute of Analytical Instrumentation). NAP-22 primers sequences and fluorescent probes (JSC «Syntol», Russia) were used. β-actin gene was used for internal control. The difference in mRNA NAP-22 expression was evaluated by Wilcoxon-Mann-Whitney test. Differences were considered significant, if the probability exceeded 95% ($n = 12$, $U = 1$).

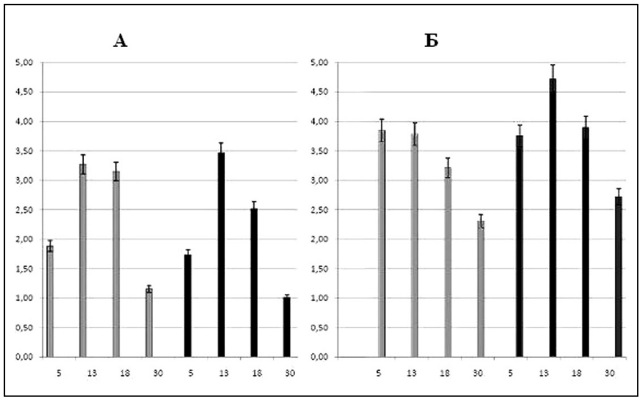
Results

In SHR rats aged 13 days, with normal calcium intake (the first group) (Fig. 1A), the level of mRNA NAP-22 expression increased in the cortex and in the medullar kidney layers. At the age of 18 days mRNA NAP-22 expression in the medullar layer was significantly lower than in the cortex, and on the 30th day it was lower than on the 5th day after birth. In WKY rats (Fig. 1B) mRNA NAP-22 expression in both layers varied similarly with SHR, but was significantly higher ($p \leq 0.05$).

In animals with calcium deficiency (second group), interline ratio of mRNA NAP-22 expression changed dramatically: in SHR rats (Fig. 2A) expression in both layers was significantly higher, and in WKY rats expression (Fig. 2B) was lower than in the first rat group (Fig. 1).

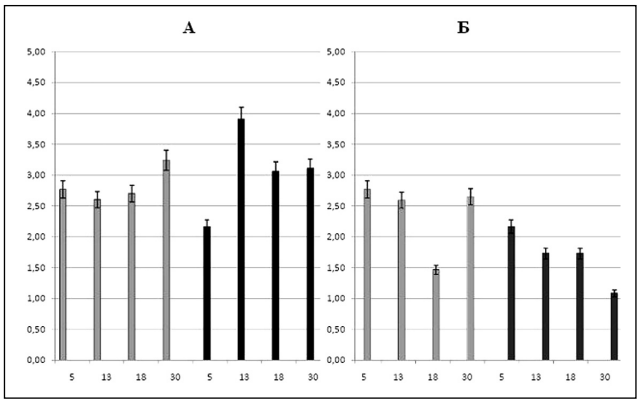
In SHR rats, number of mRNA NAP-22 copies was significantly ($p \leq 0.05$) higher in cortical layer (Fig. 3A). In WKY rats (Fig. 3B) this difference was not detected.

Figure 1. mRNA NAP-22 expression in medullar and cortical layers (group 1)



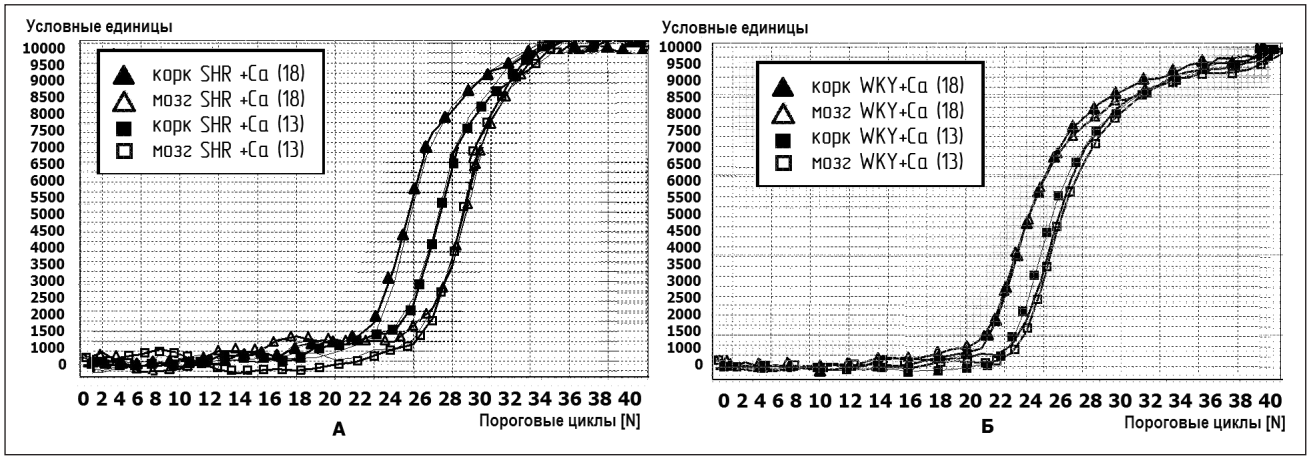
Note: SHR (А) and WKY (Б) rats of different ages. Grey column — cortical layer, black column — medullar layer. Each column represents average (M±m) result of 6 animals.

Figure 2. mRNA NAP-22 expression in medullar and cortical layers (group 2)



Note: SHR (А) and WKY (Б) rats of different ages. Grey column — cortical layer, black column — medullar layer. Each column represents average (M±m) result of 6 animals.

Figure 3. Number of mRNA NAP-22 copies in medullar and cortical layers



Note: SHR (А) and WKY (Б) rats 13 and 18 days old. Axis of abscises shows numbers of PCR threshold cycles. Axis of ordinates shows signal intensity (ID).

Therefore, in ontogenesis, interline differences in mRNA NAP-22 expression in rat kidney structures were revealed. In normotensive rats with normal calcium intake, expression was higher than in SHR rats up to 30th day, and then it decreased.

Discussion

There might exist compensatory mechanisms in normotensive rats normalizing cytoplasm calcium levels, for example effective work of sodium-calcium adenosinetriphosphatase (ATPase) [7]. In this case, temporal increase in protein kinase C (PKC)-mediated pathway activity may occur leading to the changes in mRNA NAP-22 expression.

In case of exogenous calcium deficiency, there is a dramatic change: in spontaneously hypertensive rats mRNA NAP-22 expression increases, while in WKY rats, on the contrary, it goes down.

In SHR rats this can be explained by genetically determined calcium channels abnormalities in kidney cells [8, 9] and reduced ATPase activity [7]. In normotensive rats the same calcium deficit may reduce PKC-dependent cascades and intracellular signaling that, respectively, reduces mRNA NAP-22 expression.

Therefore, changes in mRNA NAP-22 expression with different calcium intake may indicate a degree of calcium metabolic disorders in kidney cells.

Conclusions

1. Genetic calcium metabolic disorders in association with decreased Ca2+ intake cause cell dysfunction in kidney cortical layer. This is reflected by compensatory increase in mRNA NAP-22 expression.

2. An increase in mRNA NAP-22 expression can be attributed to the intracellular calcium-dependent cascade activation. In SHR rats, this reaction depends more on calcium intake, and develops long before sustained improvement in arterial vascular tone.

The role of circulating volume, filtration and reabsorbtion in HTN pathogenesis needs further investigation. In accordance with our results, they appear long before changes in vascular SMC.

Conflict of interest. The authors declare no conflicts of interest.

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