Changes of cardiovascular system in rats associated with high intake of sodium chloride

O.N. Beresneva[1], M.M. Parastaeva[1], G.T. Ivanova[2], A.G. Kucher[1], N.V. Shwed[1], I.J. Zaraysky[1], I.G. Kayukov[1], A.V. Smirnov[1]

[1] First Pavlov State Medical University of St. Petersburg, St Petersburg, Russia

[2] Institution of Physiology named after I.P. Pavlov, St Petersburg, Russia

Corresponding author: Olga N. Beresneva, MD, PhD, Senior Researcher, Laboratory of Kidney Clinical Physiology, Nephrology Institute, Pavlov First State Medical University of St. Petersburg, 6/8 L. Tolstoy street, St Petersburg, Russia, 197022. Phone: + 7(812)346–39–26. E-mail: beresnevaolga@list.ru

Abstract

Objective. The aim of the research was to study the influence of diet with different contents of NaCl on the level of arterial blood pressure (BP), processes of myocardial remodeling in response to changes in nuclear transcription factor κB (NFκB) expression in the myocardium in rats. **Design and methods.** Two groups of male Wistar rats have received a diet with normal (0,34 %; n = 8) and high (8,0 %; n = 8) content of NaCl for 2 months. BP was measured; urea, creatinine and sodium levels in blood serum and creatinine, protein and sodium levels in the urine were determined. The cardiac left ventricular mass index (LVMI) was calculated, morphological study of myocardium (lightoptical microscopy), including quantitative morphometry was carried out. The relative expression level of the NfκB gene was assessed in heart. **Results.** The high-salt diet resulted in a significant increase of diuresis and sodium concentration in the urine. High diet levels of NaCl did not affect significantly BP and LVMI. However, there were changes in the myocardium structure in the high-salt diet group, including myocardium hypertrophy and hyperplasia of cardiomyocytes, perivascular fibrosis, angiospasm, increased thickness of the artery walls due to smooth muscle cells hypertrophy, their vacuolization, and vascular sclerosis. There was a 3,4-fold increase in NFκB gene expression level in myocardium in the high-salt group compared to the low-salt group. **Conclusions.** Our findings suggest that the high consumption of sodium chloride can cause myocardial remodeling regardless of changes in BP.

Key words: cardio-vascular system, myocardial hypertrophy, sodium chloride, nuclear transcription factor κB.

Received 30.05.2014; accepted 05.07.2014.

Изменения сердечно-сосудистой системы у крыс, сопряженные с высоким потреблением хлорида натрия

О.Н. Береснева[1], М.М. Парастаева[1], Г.Т. Иванова[2], А.Г. Кучер[1], Н.В. Швед[1], М.И. Зарайский[1], И.Г. Каюков[1], А.В. Смирнов[1]

[1] Государственное бюджетное образовательное учреждение высшего профессионального образования «Первый Санкт-Петербургский государственный медицинский университет имени академика И.П. Павлова» Министерства здравоохранения Российской Федерации, Санкт-Петербург, Россия

[2] Федеральное государственное бюджетное учреждение науки «Институт физиологии имени И.П. Павлова» Российской академии наук, Санкт-Петербург, Россия

УДК.612.084:612.17

Контактная информация: Береснева Ольга Николаевна, ГБОУ ВПО «Первый Санкт-Петербургский государственный медицинский университет имени академика И.П. Павлова» Минздрава России, ул. Льва Толстого, д. 6/8, Санкт-Петербург, Россия, 197022. Тел.: +7(812)346–39–26. E-mail: beresnevaolga@list.ru

Резюме

Целью настоящего исследования стало изучение влияния рационов питания с разным содержанием поваренной соли на уровень артериального давления (АД) и процессы ремоделирования миокарда в связи с изменениями уровня экспрессии нуклеарного фактора транскрипции κB (NFκB) в миокарде у крыс. **Материалы и методы.** Исследовано две группы крыс-самцов линии Wistar, получавших пищевой рацион с нормальным (0,34 %; n = 8) и высоким (8,0 %; n = 8) содержанием NaCl в течение 2 месяцев. Оценивались АД, концентрации в сыворотке крови: мочевины, креатинина, натрия; в моче — креатинина, белка и натрия. Рассчитывался индекс массы миокарда левого желудочка (ИММЛЖ), проводилось морфологическое светооптическое исследование миокарда, включая количественную морфометрию. В ткани сердца исследовался относительный уровень экспрессии гена нуклеарного фактора транскрипции κB. **Результаты.** Содержание животных на рационе с большим количеством поваренной соли приводило к существенному нарастанию диуреза и концентрации натрия в моче. Величины АД и ИММЛЖ под влиянием высокого содержания соли существенно не изменялись. У животных с высоким потреблением NaCl развивались изменения в миокарде, выражающиеся в гипертрофии и, возможно, гиперплазии кардиомиоцитов. Кроме того, высокое потребление хлорида натрия приводило к развитию значительного периваскулярного фиброза, выраженному ангиоспазму, увеличению толщины стенки артерий за счет гипертрофии гладкомышечных клеток, вакуолизации гладкомышечных клеток и формированию перивазального склероза. Уровень экспрессии гена нуклеарного фактора транскрипции κB в ткани миокарда животных, питавшихся кормом с высоким содержанием поваренной соли, оказался в 3,4 раза выше, чем у крыс, получавших низкосолевой рацион. **Выводы.** Полученные данные свидетельствуют, что высокое потребление хлорида натрия может приводить к активизации процессов ремоделирования миокарда независимо от изменения АД.

Ключевые слова: сердечно-сосудистая система, гипертрофия миокарда, хлорид натрия, нуклеарный фактор транскрипции κB.

Статья поступила в редакцию 30.05.14 и принята к печати 05.07.14.

Introduction

Despite currently available clinical and experimental data, the question about mechanisms of the effect of high sodium chloride diet on cardiovascular and renal system is still open. There is no doubt that dietary salt restriction decreases cardiovascular morbidity and mortality [1]. At the same time a radical sodium intake restriction may lead to some negative consequences [2].

Moreover, it is suggested that high salt diet is an independent factor for remodeling and is not associated with the significant increase in blood pressure (BP). Thus, there is a different individual response to higher chloride sodium intake. Large part of the individuals with normal BP (about 25 %) with ordinary dietary salt intake (salt-sensitive normotensives) have higher risk of hypertension (HTN) onset, but their cumulative mortality index is the same as in patients with resistant HTN. Despite the ongoing research of salt sensitivity and load, the problem is not finally resolved due to the contradictory data on mechanisms, as well as definition and criteria [3]. Traditionally, high salt consumption is believed to promote water retention and extracellular fluid volume expansion, which leads to the development of volume-dependent HTN. However, in last years, new clinical and experimental data have been received. In brief, there is a direct sodium chloride «toxicity» on certain organs and tissues. Such «toxic» effect of NaCl eventually leads to microcirculatory remodeling. This mechanism implies activation of different proliferative, and pro-fibrotic pro-inflammatory cytokine signaling pathways that are associated or controlled by expression of nuclear transcription factors. Simultaneously an increase in the resistance of small skin vessels can be one

of contributing factors for BP rise, regardless of volume expansion [3, 4]. Nevertheless, specific ways of impact of high sodium chloride intake on hemodynamics and myocardial remodeling remain undiscovered.

The aim of the research was to study the influence of different NaCl consumption on BP level, myocardial remodeling in response to changes of nuclear transcription factor κB (NFκB) expression in the myocardium in rats.

Design and methods

Two groups of adult male Wistar rats («Koltushi» vivarium of Russian Academy of Sciences) were investigated. The first group consisted of 8 intact male rats fed on standard diet (0.34% NaCl) for 2 months. The second group included 8 intact adult male rats fed on high salt diet (8.0% NaCl). Both groups were fed on the same amount of proteins, fats and carbohydrates with free water access.

Blood pressure registration

Before starting the experiment, and one day before its end systemic BP was measured by cuff method in awake state. For this, animals were placed in a separate chamber, occluding cuff was put on and was connected to the "ENEMA" electrical manometer (Sweden). BP was evaluated as the cuff pressure at the time of pulse fluctuations discontinuation. Four-five BP measurements were performed in each rat, and the average value of the last three measurements was calculated. At the end of experiment all animals were anesthetized (etherization) and decapitated. All studies were conducted in accordance with International Standards for the Care and Use of Laboratory Animals and were approved by the Ethics Committee of the First Pavlov State Medical University of St. Petersburg.

Biochemistry test

Daily urine was collected and its volume was recorded (V, ml/24hrs). Blood tests, including urea (Sur, mmol/l), creatinine (Scr, mmol/l), sodium chloride (SNa, mmol/l), and urine-creatinine (Ucr, mmol/l) protein (UP, g/l) and sodium chloride (UNa, mmol/l), were performed.

Hypertrophy index estimation

After the experiment, the heart was extracted and left ventricular myocardial mass (LVM, mg) was assessed in all animals. As long as the body mass is associated with the hypertrophy, an experimental cardiology formula (LVM/body weight ratio [5]) for LVM index calculation was used (LVMI, mg/g).

NFkB gene expression investigation

A sample of rat myocardial tissue was collected in sterile conditions. The tissue was put into plastic autoclaved microtubes called «Eppendorf» 1.5 ml, adding 0.2 ml 0.1 M EDTA. Then it was homogenized by single-use blade until being pultaceous. The resulting sample was washed in PBS and TE and was used for further total ribonucleic acid (RNA) precipitation. Precipitation of total RNA was performed with phenol-chloroform, with the set «RIBO-sol-A» («AmpliSens» Russia). Complementary deoxyribonucleic acid (cDNA) preparation was conducted with a reverse transcription reaction (set «Reverte-L-100», «AmpliSens», Russia) at randomized oligopraymer modification using a reverse transcriptase M–MLV. This protocol allowed to use the resulted cDNA as a single target for post following amplification. Amplification reaction (RealTimePCR-protocol) and detection were carried out using DT-96 («DNA Technology», Russia). There were two separate reactions set up for each of 2 reactions — one for gene NFkBp65 and one for GAPDH gene, respectively. Reaction mixture («Syntol» Russia) with intercalating SYBRGREEN stain was used for polymerase chain reaction (PCR) analysis. Composition of the reaction mixture (25 μl) was the following: 2,5×10 μl reaction buffer, 2,5 μl MgCl2 (25 mM), 2,5 μl nucleotide triphosphates (2.5 mM), a pair of primers of 10 pmol/l, 0.2 μl Taq-polymerase solution 5 U/ml and 4 μl cDNA. Primers were synthesized at Research and production company «LITEH» (Moscow, Russia).

Primer sequences were the following:

NFkBp65F: 5-GTTCACAGACCTGGCATCC-3;
NFkBp65R:-TGTCACTAGGCGAGTTATAGC-3;
GAPDH-F: 5-TGGAAATCCCATCACCATCT-3;
GAPDH-R: -GTCTTCTGGGTGGCAGTGAT-3.

Reagent contamination control was carried out with the use of mandatory negative control (H2O instead of cDNA). Typical amplification program included initial denaturation — 95ºC (300 seconds) and 35 cycles (95 º C — 15 seconds, 61 º C — 40 seconds). Results were analyzed automatically in «qualitative logarithmic shortchanging» mode. Calculation of the relative NFkB gene expression was carried out by semiquantitative Protocol by 2 ΔΔSt.

Histopathology

For histopathology and immunomorphology study, myocardial samples from each animal were immediately fixed in 4 percent PFA PBS buffered solution, pH 7.4 for 24 hours at room temperature. After standard processing of the tissue fragments (dehydratation and impregnation) from the paraffin blocks, serial sections 4–5 microns thick were prepared and stained by hematoxylin/eosin and van Gieson's pirofuchsine. Light optical method was used. A severity of histopathologic changes was estimated by quantitative morphometry program VideoTest 5.2. Statistical analysis was performed using the software package Statistica 6.0. All results are presented as mean ± SEM. The differences were assessed by t-test Student for unpaired comparisons. Differences with p <0.05 were considered significant.

Results

Consumption of high salt diet within 2 months had not caused statistically significant BP increase compared with control group (Table 1). Urea, creatinine, serum sodium levels did not differ in study groups (Table 1). In contrast, urine volume and especially sodium urine concentration were larger in animals of group 2 than in animals of group 1 by the end of experiment (Table 1). In animals treated with 8.0 % NaCl-containing diet, urine-creatinine concentration significantly reduced. There was no noticeable high salt intake effect regarding the severity of proteinuria. LVM and LVMI did not significantly differ in 2 groups (Table 1). At the same time there were significant differences in myocardial morphology results in rats fed on different amounts of dietary salt. High salt diet led to nuclear polymorphism and hypertrophy of cardiomyocytes, loss of cross striation, protein degeneration and cytoplasm lumpy decay of muscle fibers. Moderate intermuscular edema was found (Fig. 1). Perivascular fibrosis was observed in myocardial sections in control group (probably due to the fact that adult rats were included in the study). High salt diet resulted in an abrupt rise of perivascular fibrosis and vasospasm along with the hypertrophy of cardiomyocytes (Fig. 1).

High salt diet also caused growth of artery wall thickness due to smooth muscle cells hypertrophy,

Table 1

ANIMAL CHARACTERISTICS AT THE END OF THE STUDY

Parameter X ± m	Group 1 (n = 8)	Group 2 (n = 8)	p
BP, mmHG	130.0 ± 5.0	135.0 ± 5.0	> 0.05
Body mass of rat, g	358.0 ± 11.5	324.7 ± 14.6	= 0.096
LVM, mg	820.7 ± 24.0	746.7 ± 30.0	= 0.077
LVMI, mg/g	2.30 ± 0.04	2.31 ± 0.07	> 0.05
Scr, mmol/l	0.044 ± 0.01	0.038 ± 0.04	> 0.05
SNa, mmol/l	143.8 ± 1.2	144.1 ± 1.0	> 0.05
Sur, mmol/l	6.2 ± 0.5	5.6 ± 0.8	> 0.05
V, ml/ 24h	7.0 ± 0.5	8.9 ± 1.0	< 0.05
UNa, mmol/l	86.26 ± 1.0	401.5 ± 60.21	< 0.001
Ucr, mmol/l	13.04 ± 2.16	8.0 ± 1.8	< 0.01
UP, g/l	0.88 ± 0.17	0.61 ± 0.24	> 0.05

Note: BP — blood pressure, LVM — left ventricular mass, LVMI — left ventricular mass index, Scr — cerum creatinine, SNa — serum sodium, Sur — serum urea, V — urine volume, UNa — urine sodium, Ucr — urine sodium, UP — proteinuria.

**Figure 1. Morphological cardiomyocyte changes.
А — low salt diet (control group), Б — high salt diet.
Hematoxylin-eosin staining × 100**

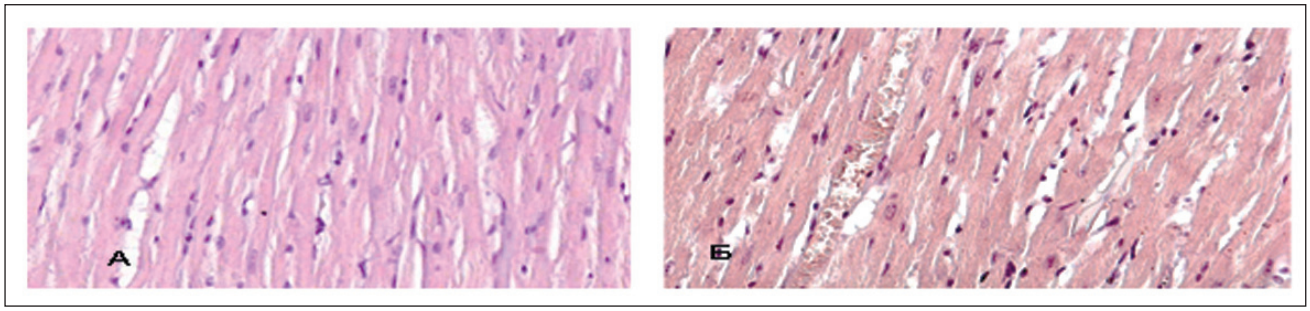

**Figure 2. Morphological vascular changes.
А — low salt diet (control group), Б — high salt diet.
Van Gieson's stain × 200**

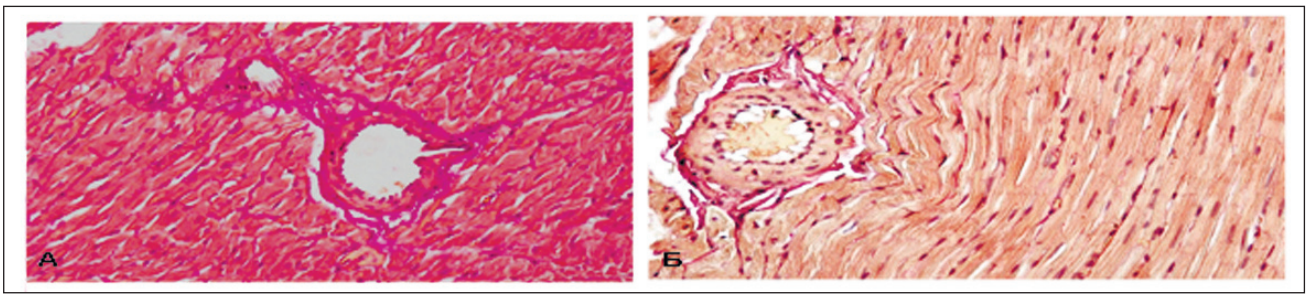

Figure 3. Relative myocardial gene NFkB expression level (80 — group 1, 81 — group 2)

	Number of the rat							Mean values	Relative myocardial NFkB gene expression	
	80_1	80_2	80_3	80_4	80_5	80_6	80_7	80,0	ΔΔCt	80/81
NFkBp65	31,0	32,6	33,1	31,6		34,4	31,9	32,4	1,8	0,3
GAPDH	31,4	31,5	29,2	25,6	26,2	29,3	26,1	28,5		
	81_1	81_2	81_3	81_4	81_5	81_6		81,0		81/80
NFkBp65	30,0	33,7	31,6	32,3	33,8	32,3		32,3	-1,8	3,4
GAPDH	32,1	26,0	33,2	31,7	29,1	28,4		30,1		

Table 2

QUANTITATIVE MORPHOMETRY IN CARDIOMYOCYTES IN HIGH SALT DIET GROUP

Paramete X ± m	Groups		
	Group 1	Group 2	P
Cardiomyocyte thickness, mkm	11.07 ± 0.4	14.1 ± 0.3	< 0.001
Nucleus area, mkm²	36.2 ± 2.3	44.3 ± 1.7	< 0.02
Nucleus length, mkm	12.6 ± 0.6	15.0 ± 0.6	< 0.02
Nucleus thickness, mkm	3.7 ± 0.2	3.9 ± 0.1	Statistically non-significant

smooth cell vacuolation and perivascular sclerosis (Fig. 2).

Quantitative morphometry showed that high salt diet led to the cardiomyocyte thickening and the increment of length and area of myocardial nuclei (Table. 2).

Lastly, the level of gene expression of nuclear κB transcription factor in myocardial tissue was 3.4-times higher in high salt diet group than in rats fed on low salt diet (Fig. 3).

Discussion

Some results in our research were quite unexpected. We have not found a significant increase in BP in rats fed on high salt diet for 2 months. There was an increase in renal sodium excretion (Table 1) mainly due to the tubular reabsorption inhibition. There was more than 1.2-fold increase in urine volume and 5-fold rise in UNa (Table 1). There was no significant changes in glomerular filtration rate in high salt diet animals (slight diuresis incremental with relatively low Ucr) (Table 1). Wistar rats have a high capacity to eliminate sodium chloride. Probably, there was no significant blood volume expansion during the study, which could serve as one of the reasons for BP increase. One of the possible BP increment mechanisms associated with high NaCl intake (total peripheral vascular resistance growth related to lymph-capillary remodeling in skin vessels) did not work either. The possible mechanism might be the interstitial tone increment stimulating infiltrating macrophages to express TonEBP — transcription factor that increases the production of vascular endothelial growth factor C (VEGF-C). Also an endothelial form of NO synthase (NOS3) was activated in high salt diet group, which was partially mediated by transforming growth factor beta (TGF-β). It is an additional way for sodium ions depositing and volume expansion prevention. Moreover, the activation of NOS3 leads to the nitric oxide synthesis preventing BP elevation. Salt-sensitive HTN is partially explained by impairment of this mechanism [3, 4]. We can assume that this and similar compensatory mechanisms are sufficiently reliable in laboratory animals and can prevent HTN development despite significantly long high consumption of dietary salt.

At the same time, high salt diet affects cardiovascular system. LVMMI was equal in both groups. Therefore pathomorphology including quantitative morphometry results suggest that distinct manifestations include hypertrophy and possibly hyperplasia of cardiomyocytes in rats consuming a diet with 8.0% NaCl (Table 2, Fig. 1). Herewith nuclear transcription factor κB gene expression clearly increases in myocardium in this group of animals (Fig. 3). Altogether these data suggest that high salt diet leads to a specific myocardial remodeling beyond BP rise in normotensive Wistar rats. There should be compensatory mechanisms in cases of myocardium hypertrophy development (contraction of fat or connective tissue?), but there is no answer to this question yet. Remodeling of myocardial small vessels (Fig. 2) may cause further heart remodeling. These changes might be associated with NFkB-associated signaling pathways activation. However, their role in this type of myocardial remodeling requires further investigation.

Conclusions

Our findings suggest that 2-month high salt diet did not lead to a significant increase in BP and LVMI in Wistar rats. Peculiar changes, including hypertrophy and possibly hyperplasia, were found in cardiomyocytes. Additionally, high sodium chloride intake leads to the development of significant perivascular fibrosis, manifesting angiogenic spasm, increased arterial wall thickness due to the smooth muscle cells hypertrophy, vacuolation, and perivascular sclerosis. We suggest that these changes are related to the NFκB-associated signal ways activation.

Disclosure. The authors declare that there are no conflicts of interest.

References

1. Klaus D, Hoyer J, Middeke M. Salt restriction for the prevention of cardiovascular disease. Dtsch. Arztebl. Int. 2010;107(26):457–462.

2. Ritz E, Mehls O. Salt restriction in kidney disease — a missed therapeutic opportunity? Pediatr. Nephrol. 2009;24 (1):9–17.

3. Kanbay M, Chen Y, Solak Y, Sanders PW. Mechanisms and consequences of salt sensitivity and dietary salt intake. Curr. Opin. Nephrol. Hypertens. 2011;20(1):37–43.

4. Pase MP, Grima NA, Sarris J. The effects of dietary and nutrient interventions on arterial stiffness: a systematic review. Am. J. Clin. Nutr. 2011;93(2):446–454.

5. Okoshi K, Ribeiro H, Okoshi M et al. Improved systolic ventricular function with normal myocardial mechanics in compensated cardiac hypertrophy. Jpn. Heart.J. 2004; 45(4):647–656.