PHYSIOLOGY

Effect of Exogenous Calcium Deficit on Blood Pressure and Modification of Brain Proteins GAP-43 and BASP1 in SHR and WKY Rats

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We studied the effect of drinking of low mineralized water containing 8 mg/liter Ca^{2+} on blood pressure and content of brain proteins in synaptosomes of SHR and WKY rats. Blood pressure increased in WKY rats, but not in SHR rats. In SHR rats, Ca^{2+} deficit reduced the content of GAP-43 protein and induced the appearance of its fragment GAP-43-3 in brain synaptosomes. In WKY rats, the content of this protein did not change, and its fragment GAP-43-3 was absent. No structural changes in BASP1 protein were found.

Key Words: *calcium; SHR; WKY; arterial hypertension; first terminal proteins*

Deficit of exogenous Ca^{2+}, *e.g.* its low content in drinking water, plays a key role in the pathogenesis of increased blood pressure (BP) in spontaneously hypertensive SHR rats [14]. We also found that in normotensive Wistar—Kyoto (WKY) rats usually used as the control in studies on SHR rats, BP increases if they drink water with low content of bioavailable and bioactive Ca^{2+} pool for a long time [2].

SHR rats are characterized (apart from hypertension) by pronounced cognitive disturbances [7] progressing with age. It can be assumed that they can be caused by hypoperfusion of tissues, tissue hypoxia, and other negative consequences of increased tonus of blood vessels supplying the brain [6,12]. Recently, special attention in studies of disturbances of brain neurons was attracted to the effect of various factors modulating molecular processes accompanying maturation of neurons and formation of neuronal networks.

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Key regulatory proteins of nerve terminals GAP-43 (B-50, neuromodulin) and BASP1 (CAP-23, NAP22) are peculiar marker of plastic processes in the nervous system [5]. These proteins play an important role in neurite growth and navigation of the growth cones during the development of the nervous system. These proteins are probably the target for exogenous Ca^{2+}, especially during the embryonic and early postnatal development, when the expression of these factors attains maximum because of their involvement into neuritogenesis and synaptogenesis.

Therefore, in parallel with parameters of hemodynamics in adult animals we studied structural modifications of GAP-43 and BASP1 proteins in postnatal periods in spontaneously hypertensive (SHR) and normotensive (WKY) rats under conditions of normal and reduced Ca^{2+} supply.

MATERIALS AND METHODS

The experiments were carried out on male and female SHR and WKY rats. Two groups for each strain

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were formed (A and B, 8 animals per group). The animals received standard pellet providing normal daily amount of Ca^{2+}. Group A animals received water containing 80 mg/liter Ca^{2+}, which corresponded to WHO requirements. Group B animals after transition to definitive nutrition (at the age of 4-6 weeks) received low mineralized water containing 8 mg/liter Ca^{2+}.

The animals were maintained under these conditions for 90 days, then they were mated, and the progeny was obtained. During pregnancy and nursing, group A dams received normally mineralized water, and group B dams received low mineralized water. The rat pups were decapitated after 18 days. This period of postnatal development was chosen because cell structures of the hippocampus are completely formed at this term [13].

The brain was removed and the nervous tissue was immediately fractionated using a method of isolation of crude synaptosome fraction enriched with GAP-43 and BASP1 proteins. The proteins were than purified using routine methods [8]. The content of GAP-43 and BASP1 proteins in samples was determined by surface immunoblotting [10] after electrophoresis in 12% PAAG using 0.9 M acetic acid+2.5 M urea system. The gels were stained with 0.1% Coomassie R250 in a solution containing 25% isopropanol and 10% acetic acid. The proteins were transferred from the gels onto nitrocellulose membrane filters (Synpor). The immunoblots were developed using antibodies to GAP-43 protein and A protein conjugated with horseradish peroxiadse (Pasteur Research Institute of Epidemiology and Microbiology).

Before sacrifice, some animals were isolated and after transition to definitive nutrition, they were maintained under the same conditions as their parents. Starting from the age of 10-12 weeks, systemic BP in the caudal artery was measured in some alert animals (body weight 180-200 g) by an indirect tachooscillographic method. The mean BP was calculated from three consecutive measurements.

The data were processed statistically using Student *t* test.

RESULTS

Changes in cationic composition (Ca^{2+} content) of drinking water produced different effects of the dynamics of BP in spontaneously hypertensive and normotensive rats (Fig. 1). The mean BP in both groups of male WKY rats was lower than in SHR rats, but in group B it increased to 144.3±3.5 mm Hg, while in group A it was normal (125.5±3.2 mm Hg; $p<0.05$).

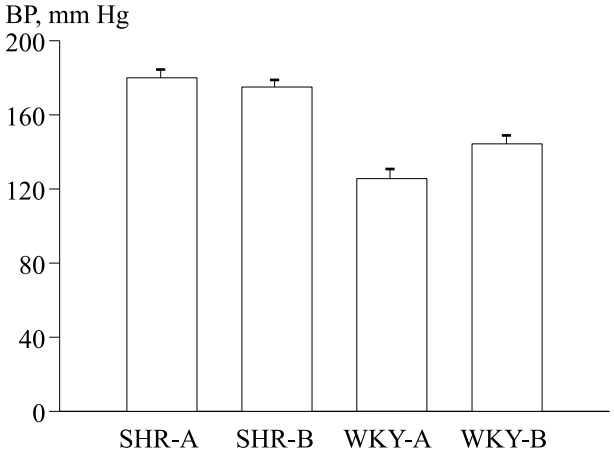

Fig. 1. Effect of Ca^{2+} deficiency on mean BP in SHR and WKY rats.

No intergroup differences were found between male SHR rats of groups A and B: the mean BP in the caudal artery was 180.0±4.6 and 175.0±4.6 mm Hg, respectively.

The absence of further increase in BP in SHR rats against the background of exogenous Ca^{2+} deficit can be explained by the presence of both functional abnormalities and changes in molecular composition of some channels of the cytoplasmic membrane (L-calcium channels and BK channels) in vascular smooth muscle cells (SMC) in genetic models of pronounced arterial hypertension, which are absent in normotensive rats [4]. These changes lead to accumulation of Ca^{2+} ions in SMC cytosol; and these disturbances are pronounced and cannot be modified by variations in cationic composition of drinking water.

Deficit of exogenous Ca^{2+} leads to accumulation of intracellular Ca^{2+} in the sarcoplasm of vascular SMC and cardiomyocytes [10]. These changes can be the cause of borderline hypertension in normotensive rats of inbred strains under conditions of restricted Ca^{2+} consumption.

It was found that deficit of exogenous Ca^{2+} affects not only the state of vascular SMC, but also functioning of neurons and formation of neuronal

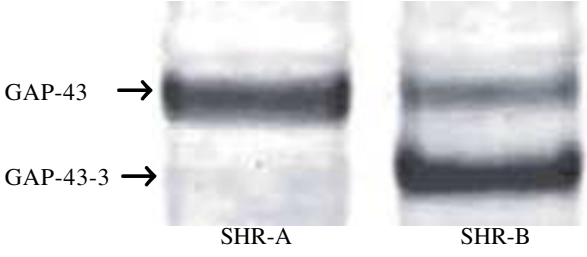

Fig. 2. Distribution of GAP-43 protein and its fragment GAP-43-3 in the fraction of synaptosomes in SHR rats. Immunoblots were developed using polyclonal antibodies to GAP-43 protein.

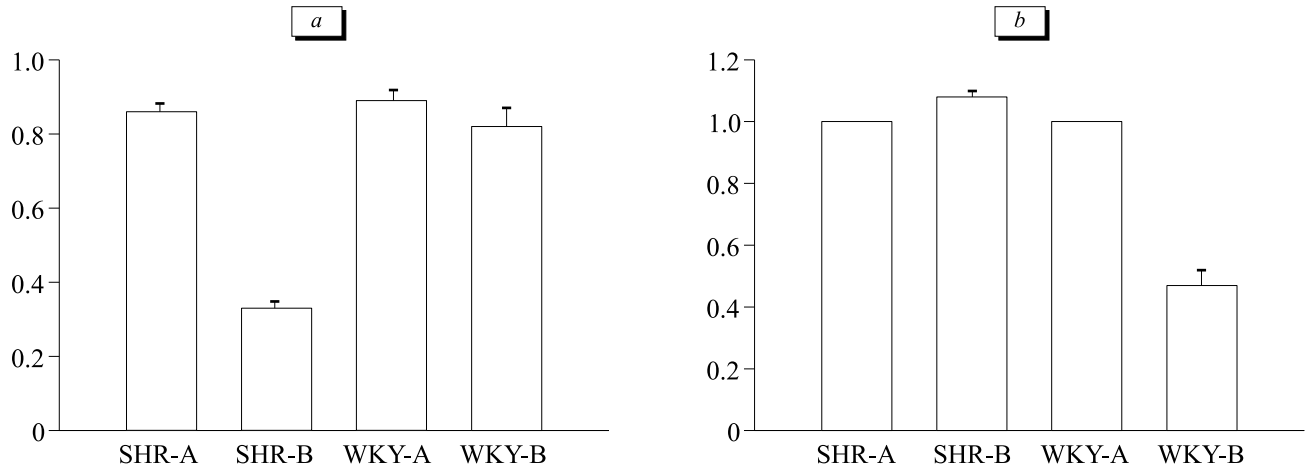

Fig. 3. Percent of GAP-43 (*a*) and BASP1 (*b*) proteins in synaptosomes of SHR and WKY rats. *a*) total content of GAP-43 and GAP-43-3 is taken as 1; *b*) protein content in synaptosomes of group A rats.

networks during the early ontogeny. In our experiments, drinking of low mineralized water was associated with the appearance of not only GAP-43 protein (detected by specific staining) in synaptosomal fraction, but also its fragment GAP-43-3 (Fig. 2, *a*). In SHR-A animals this fragment was practically absent in all examined samples (Fig. 2, *b*), while the content of GAP-43 protein was considerably higher. In synaptosomes of SHR-B rats, the relative content of GAP-43 protein in the total fraction of GAP-43 and GAP-43-3 proteins was lower (by 53±2%, $p<0.01$) than in rats receiving water with normal Ca^{2+} content (Fig. 3, *a*). The content of GAP-43 protein in synaptosomal fraction of the brain from WKY rats was similar in the two subgroups, and none samples contained GAP-43-3 fragment (Fig. 3, *a*). The content of BASP1 protein in synaptosomes of SHR-A rats was higher (by 53±5%; $p< 0.01$) than in SHR-B rats (Fig. 3, *b*). The content of BASP1 protein in synaptosomes of WKY-A and WKY-B rats was similar (Fig. 3, *b*).

These findings can be explained as follows. It is known that borderline hypertension in WKY rats little affects their cognitive function, whereas SHR rats are characterized by poorer learning compared to WKY rats and SHR rats receiving Ca^{2+} blockers [6]. This phenomenon can be caused by disturbed distribution and functioning of BASP1 and GAP-43 proteins. Previous studies showed that the presence of GAP-43-3, a fragment of GAP-43 protein lacking 40 N-terminal amino acid residues, can be explained by Ca^{2+}-dependent proteolysis of GAP-43 with protease calpain participating in the maintenance of synaptic plasticity and cell death [1]. Proteolysis of GAP-43 protein *in vitro* proceeds with high rate only in the presence of high Ca^{2+} concentrations [9].

Thus, cognitive disturbances in SHR rats cannot be explained exclusively by hypoperfusion of the brain. The effect of exogenous Ca^{2+} deficiency on neuronal function during ontogeny in these rats is realized via a more complex pathway involving processes of neuronal maturation, formation of neuronal contacts, and expression of certain enzymes.

Our findings suggest that the effects of exogenous Ca^{2+} deficiency on the formation of arterial hypertension and cognitive disturbances in rats of both strains (SHR and WKY) can be realized via independent mechanisms.

REFERENCES

1. V. V. Zakharov, M. N. Bogdanova, and M. I. Mosevitskii, *Biokhimiya*, **70**, No. 8, 1086-1098 (2005).
2. D. B. Ryzhov, N. Z. Klyueva, S. K. Churina, and G. T. Eschanova, *Fiziol. Zh*., **79**, No. 8, 104-107 (1993).
3. D. B. Ryzhov, N. Z. Klyueva, S. K. Churina, and S. V. Kulikov, *Byull. Eksp. Biol. Med*., **117**, No. 5, 476-477 (1994).
4. G. C. Amberg and L. F. Santana, *Circ. Res*., **93**, No. 10, 965-971 (2003).
5. L. I. Benowitz and A. Routtenberg, *Trends Neurosci.*, **20**, No. 2, 84-91 (1997).
6. G. Diana, *Behav. Brain. Res.,* **134**, No. 1-2, 113-121 (2002).
7. G. Hussein, M. Nakamura, Q. Zhao, *et al., Biol. Pharm. Bull*., **28**, No. 1, 47-52 (2005).
8. A. Meneses and E. Hong, Hypertension, **31**, No. 4, 968-972 (1998).
9. M. I. Mosevitsky, J.-P. Capony, G. Yu. Skladchikova, *et al., Biochimie*, **79**, No. 6, 373-384 (1997).
10. S. N. Orlov, J.-M. Li, J. Tremblay, P. Hamet, *Semin. Nephrol.*, **15**, No. 6, 569-592 (1995).
11. A. Y. Plekhanov, *Anal. Biochem*., **239**, No. 1, 110-111 (1996).
12. R. Prediger, F. A. Pamplona, D. Fernandes, R. N. Takahashi, *Int. J. Neuropsychopharmacol*., **8**, No. 4, 583-594 (2005).
13. R. Rice and S. Barone, *Environ. Health Perspect*., **108**, Suppl. 3, 511-533 (2000).
14. R. Schleiffer and A. Gairard, *Semin. Nephrol*., **15**, No. 6, 526-535 (1995).