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Stress plays provoking role in hypertension-related stroke: Injuries of blood-brain barrier function

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ABSTRACT

Chronic hypertension itself does not cause stroke but significantly decreases the resistant to stroke induced by stress due to exhausting of adaptive capacity of cerebral endothelium and decrease resistance of blood-brain barrier to stress.

Keywords: hypertension, stroke, stress, blood-brain barrier

1. INTRODUCTION

Every year, 15 million people worldwide suffer a stroke. Nearly six million die and another five million are left permanently disabled. Stroke is the second leading cause of disability, after dementia. One of six people worldwide will have a stroke in their lifetime. Every six seconds, stroke kills some. The mechanisms underlying stroke still remain little understood. Hypertension is the most prevalent of risk factors for stroke but its direct role in stroke is not generally appreciated.[1,2] Despite the substantial evidence of the benefits of lowering blood pressure, conventional treatment does not normalize the burden of major cardiovascular events in patients with hypertension. Thus, we do not have clear evidence that chronic high blood pressure itself can be provoking factor for vascular catastrophes in the brain. Fully understanding the factors involved in the hypertension-induced stroke helps to develop new strategies for stroke prevention.

There is a hypothesis that chronic dysfunctional stress plays an important role in hypertension-related stroke.[3] In our previous works on rats, we showed that stress causes vascular catastrophes in the brain through increase in permeability of blood-brain barrier (BBB) and cytotoxic brain oedema.[4–6] Our finding are correlated with data of others who showed that acutely elevated arterial pressure provokes brain microvascular permeability by diminution of claudins genes transcription in the endothelial cells of BBB.[7] These authors also showed that short-term hypertension impairs the antioxidant defense system of brain and induces brain injury through oxidative stress.[7]

Here we tested our idea that chronic high blood pressure decreases the adaptive capacity of cerebral endothelium and blood-brain barrier (BBB) to stress,[8,9] which can provokes stroke due to the increase in permeability of BBB in hypertensive rats.

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2. MATERIAL AND METHODS

Experiments were carried out in normotensive (n=73) and hypertensive (n=93) adult male rats. All procedures were performed in accordance with the "Guide for the Care and Use of Laboratory Animals". The experimental protocol was approved by the Committee for the Care and Use of Laboratory Animals at Saratov State University (Protocol H-147, 17.04.2001).

To induce hypertension we used model of chronic social stress (overpopulation). The rats must be held in a cage with the following ratio between size of cage and animal's body weight: cm^2/g of bodyweight = 1. In this model animals live in a high population density, coefficient was equal to 0.3, i.e. 3 times higher than in normal conditions.

The rats were instrumented with polyethylene catheters for monitoring of mean arterial pressure. For implantation of catheters, rats were anesthetized with isoflurane. Polyethylene catheter (PE-50 with a PE-10 tip, Scientific Commodities INC., Lake Havasu City, Arizona) was inserted into the left common carotid artery. In addition, the left femoral vein was catheterized with PE-50 tubing fused PE-10 for drug infusion.

To induce stroke we used severe intermittent sound stress (120 dB, 370 Hz) during 2h.[4–6] To confirm the development of stroke, all newborn rats were decapitated for a histological study of brain tissue. The samples were fixed in 10% buffered neutral formalin. The formalin fixed specimens were embedded in paraffin, sectioned (4 μm) and stained with haematoxylin and eosin.

To assess the changes in cerebral blood flow (rCBF or perfusion) we used a home-made system for laser speckle contrast imaging (LSCI). The raw speckle images were recorded under the following conditions: light source – HeNe laser with the wavelength 632.8 nm; image sensor – CMOS camera Basler acA2500-14gm; imaging lens – Computer M16140-MP2 16 mm at F-number equal to 6, that corresponds to speckle/pixel size ratio of around 2; exposure time – 20 ms. The speckle images were recorded for 3 min at an average frame rate of 40 frames per second. Spatial speckle contrast was calculated as $K = \sigma / < I >$, where σ is the standard deviation of intensity fluctuations and $< I >$ is the mean intensity within a 5×5 sliding window. Fifty consecutive frames were averaged into one speckle contrast image.

The permeability of BBB we studies using intravenous injection of dextran (70 kDa, confocal analysis) and gadolinium (magnetic resonance imaging (MRI), Clin Scan 7T, Bruker Biospin). The results were presented as mean ± standard error of the mean (SEM). Differences from the initial level in the same group were evaluated by the Wilcoxon test. Intergroup differences were evaluated using the Mann-Whitney test and ANOVA-2 (post hoc analysis with the Duncan's rank test). The significance levels were set at p <0.05 for all analyses.

3. RESULTS AND DISCUSSION

In the first step of our work, we analyzed the changes in BP in rats living during 4 months under social stress (overpopulation). The chronic stress was accompanied by gradual development of hypertensive status in all rats. The first month of experiment was not characterized by any changes in BP (108±3 mmHg in experimental group and 101±2 mmHg in control group). In second, third and fourth months, social stress was associated with the gradual increase in number of rats with high blood pressure (134±5, p <0.05; 141±3, p <0.05, 157±4, p <0.05 vs. 101±2 mmHg, respectively) (Fig. 1). Thus, the higher level of BP and the largest number of rats with high BP we found in forth month of social stress in rats. Notice, the placing of hypertensive rats in normal condition didn't recover BP to normotensive units suggestion about persistent increase in BP.

There results clearly show that chronic stress plays a potentiating role in hypertension formation. Considering that the average lifetime of rats – 24-30 months, 4 months of living animals under social stress is 1/6-1/7 of their life. In relation to human, it is 10–15 years of our life. Our results are consistent with those of other researchers, which suggests that long-term social stress, including emotional stress at work, family conflicts, etc., accompanied by the development of hypertension and may be regarded as a key reason for the genesis of the disease.[10, 11]

Using LSCI, we studied the changes in CBF in hypertensive rats compared with normotensive ones. LSCI data showed ischemic evens in cortex (the decrease in CBF by 17% vs. the control, p <0.05) in all hypertensive rats (Fig. 2). The optical results were confirmed by histological analysis of animal brain, when we also found

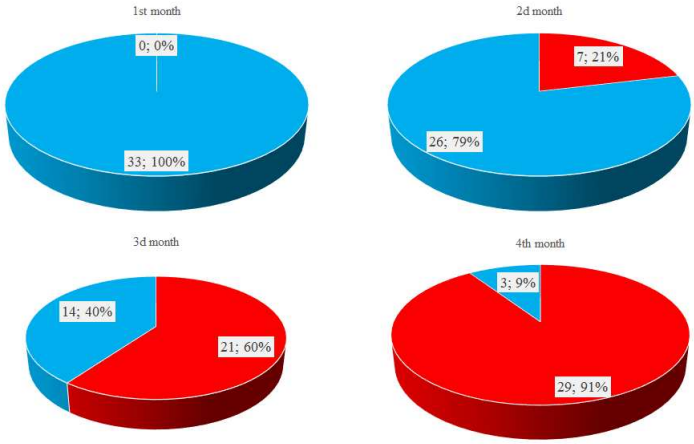

Figure 1. The ratio of rats with normal (blue) and high (red) blood pressure during 4 months of living rats under social stress (overpopulation)

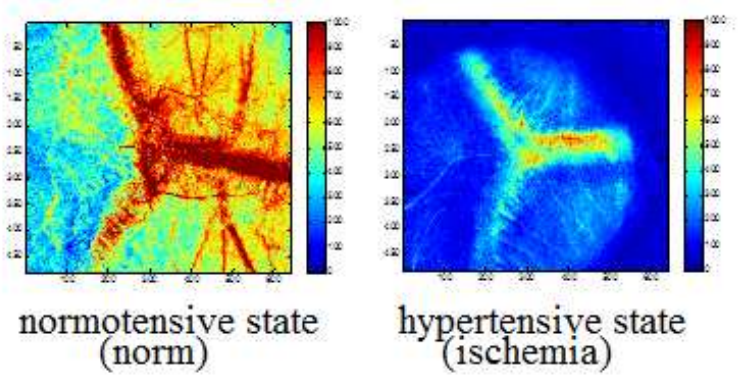

Figure 2. Laser speckle imaging of the sagittal sinus and cerebral vessels surrounding it in normotensive (in the left) and hypertensive (in the right) rats

cerebral ischemia of cortex and subcortical area of the rat brain. But, we did not find rupture of cerebral vessels in no one hypertensive animals. These results show that persistent high blood pressure itself does not cause stroke. There is opinion that not hypertension but inappropriate antihypertensive usage contributes significantly to stroke.[2]

The dextran (70 kDa) (confocal analysis) and gadolinium (MRI studies) did not permeate BBB in rats with masked (1 month of social stress) and chronic (2–4 months of social stress) stages of hypertension.

Since we didn't observe stroke in rats with chronic and persistent high blood pressure, in the next step of our investigations we used model of severe sound stress to induce stroke in hypertensive rats (n=23, after 4 months of social stress) and analyzed the changes in permeability of BBB in the pre- and post-stroke period.[4,5]

The histological analysis showed that stroke occurred in 86% (20 of 23) of hypertensive but not in normotensive rats. The pre-stroke is characterized by increase in permeability of BBB to gadolinium (552 Da) that was associated with perivascular edema in the brain of hypertensive rats (Fig. 3).

In the post-stroke time, additionally dextran (70 kDa) permeated BBB that was accompanied by formation of more severe perivascular edema (Fig. 4). Thus, in pre-stroke period stress injuries permeability of BBB to low molecular weight compounds that is associated with fluid pathway from the vessels and formation of toxic edema. In the post-stroke time, these changes were accompanied by increase in permeability of BBB to high molecular weight substances such as dextran (70 kDa).

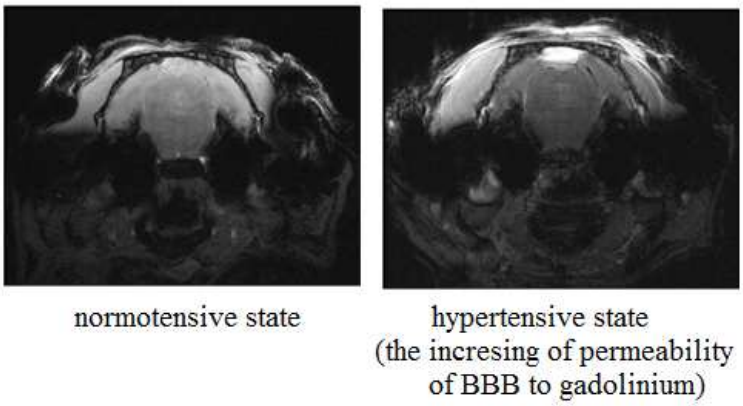

Figure 3. Magnetic resonance imaging of intensity of signal after intravenous injection of gadolinium

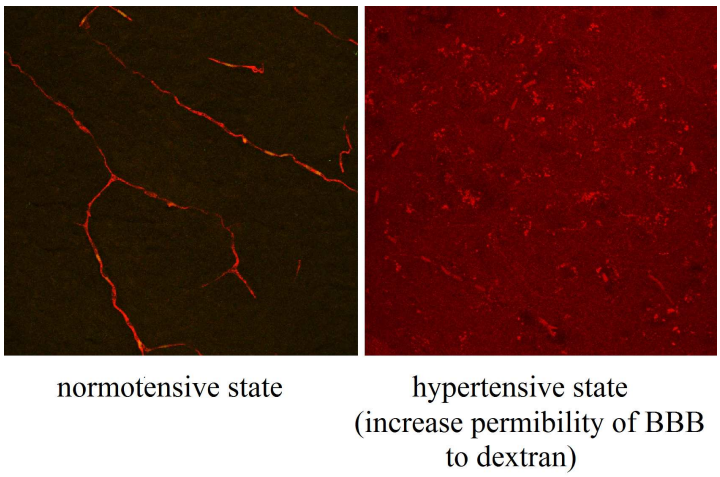

Figure 4. The confocal imaging of dextran (70 kDa, iv) in normotensive rat (dextran is inside of cerebral vessels) and in hypertensive rats after stress-induced stroke (dextran permeated BBB)

4. CONCLUSION

In summary, we conclude that chronic hypertension itself does not cause stroke but significantly decreases the resistant to stress-induced stroke due to exhausting of adaptive capacity of cerebral endothelium and decrease resistance of blood-brain barrier (BBB) to stress, that others also showed in their works.[8,9]

ACKNOWLEDGMENTS

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