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Changes of the sympathetic activity in the heart and vessels in the development of experimental vasorenal hypertension (2 kidneys — 1 clip)

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Abstract

Objective. To evaluate the dynamics of arterial blood pressure and sympathetic activity in the male Wistar rats within 8 weeks after renal artery clamping (model «2 kidneys — 1 clamp»). **Design and methods.** The sympathetic activity was examined by spectral analysis of the heart rate variability in the non-anaesthesized rats. The sympathetic vasomotor activity was examined by the registration of electric activity of the cervical spine cord in anaesthesized animals. **Results.** We found that sympathetic activity to the heart and blood vessels is comparable. Two weeks after renal artery clamping the activity of the sympathetic nervous system increases, and 4 weeks later it comes close to the reference values in the developed hypertension and then increases to maximum values by the end of the experiment. **Conclusions.** Based on our results, the mechanism of hypertension after renal artery clipping, is fairly complex, but, in the end, it appears to be associated with the increased activity of vasomotor spinal cord neurons.

Key words: vasorenal hypertension, sympathetic activity, heart rate, spectral analysis

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Характер изменения симпатической активности к сердцу и сосудам при развитии экспериментальной вазоренальной гипертензии (2 почки — 1 зажим)

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Резюме

Цель настоящего **исследования** заключалась в изучения динамики артериального давления и активности симпатической нервной системы у крыс линии Wistar на протяжении первых 8 недель развития вазоренальной гипертензии в модели «2 почки — 1 зажим». **Материалы и методы.** Симпатическую активность к сердцу оценивали на бодрствующих животных методом спектрального анализа вариабельности сердечного ритма. Симпатическую вазомоторную активность оценивали путем регистрации электрической активности шейного симпатического ствола под наркозом у тех же крыс. **Результаты.** Оказалось, что динамика симпатической активности к сердцу и сосудам принципиально не различается. При этом обнаружено, что если через 2 недели после наложения зажима на почечную артерию активность симпатической нервной системы увеличивается, то через 4 недели она приближается к контрольным значениям при развитой гипертензии и затем снова возрастает до максимальных значений к концу эксперимента. **Выводы.** Таким образом, механизм артериальной гипертензии, возникающей после клипирования почечной артерии, имеет достаточно сложный характер, однако в конечном счете основное значение, по-видимому, имеет усиление активности вазомоторных нейронов спинного мозга.

Ключевые слова: вазоренальная гипертензия, симпатическая активность, частота сердечных сокращений, спектральный анализ

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Introduction

Increased activity of the renin-angiotensin system is one of the reasons for renovascular hypertension [1, 2]. Enhanced sympathetic activity is another contributing factor [3–10]. Efferent renal nerve activity influences the sodium excretion in the renal tubules and thus participates in the long-term regulation of blood pressure (BP) [11, 12]. Changes in the afferent stimulation from the kidney with stenotic artery play an essential role in high BP maintenance in renal ischemic disease [13, 14].

However, the evidence regarding the role of sympathetic activity in renovascular hypertension development are controversial. Thus, in rabbits with hypertension 3 and 6 weeks after renal artery clipping, sympathetic activity was decreased in the non-ischemic kidney (model "two kidney — one clip") [15]. Only few studies reported increased sympathetic activity assessed by muscle neurography and norepinephrine spillover in the whole body in patients with renovascular hypertension [16].

Thus, although the increase in sympathetic activity in the clamped renal artery was proven in multiple studies, its pathogenetic role for the development and maintenance of renovascular hypertension in a model "two kidney — one clip" remains disputable. There is no agreement whether all sympathetic neurons are excited by renal ischemia, or multi-directional changes in the activity of these neurons occur. The correlations between neuron activity of the sympathetic nervous system regulating different functional elements of the cardiovascular system and BP with the course of renovascular hypertension are understudied.

The aim of our work is to study the dynamics of the sympathetic nerve activity to the heart and blood vessels in the model of renovascular hypertension "two kidneys — one clip".

Design and methods

Terms and experimental protocol

Altogether 42 male Wistar rats weighing 200–240 g were included. They were kept under free access to food and water. Terms and conditions of the study were consistent with the Ethics Committee of the Center; the protocol was approved (№ 77, 21.06.2010).

The animals were divided into 2 groups — an experimental (32 rats with hypertension developed in the model "two kidneys — one clip"), and a control one consisted of 10 animals. Before clipping the renal artery systolic BP, intersystolic interval and the instantaneous values of the intersystolic interval were recorded in rats in awake state by a non-invasive method. Heart rate variability was evaluated by fast Fourier transformation. Then, the clipping of renal artery was performed in order to model renovascular hypertension. Then, 2, 4, 6 and 8 weeks later 8 animals from each group were selected for the repeated measurements of systolic BP, intersystolic interval and the instantaneous values of the intersystolic interval, In addition, electrical activity of the sympathetic nerve was measured in anesthetized animals. In the control group, systolic BP, intersystolic interval and the instantaneous values of the intersystolic interval were also recorded 2, 4, 6 and 8 weeks later in awake state, but electrical activity of the sympathetic nerve was measured only after 8 weeks in anesthetized animals.

A model of hypertension

To create the model of renovascular hypertension, a hard tantalum clamp with 0.3 mm lumen (Kent Scientific Corporation) was fixed on the left renal artery of anesthetized rats (combination anesthesia: inhalation of ether and a solution of sodium oxybutyrate intraperitoneally 1–1.5 g/kg). Afterwards, the wound was sutured. In control (10 rats) animals the same operations were performed under general anesthesia, but the renal artery was not clipped.

Experiments in the awake state

During all the experiment, the rats had free access to food and water, the ratio day/night was 12/12 hours. For 8 weeks, every 2 weeks after renal artery clipping the examinations for the control of renovascular hypertension were performed by non-invasive measurements of systolic BP and intersystolic interval between the tail in awake rats (device ADInstruments Pty Ltd, which includes a tail-clamping cuff and a pulse sensor MLT125R). Spectral analysis of heart rate variability was performed to evaluate the autonomic regulation of cardiac activity. For this, 60-second recording episodes were selected. Spectral analysis of heart

rate variability was performed with the use of the program Chart 4.1.2 (ADInstruments). According to the methods approved by the North American Society of Pacing and Electrophysiology [17, 18], calculations were carried out in ms^2/Hz in low-frequency (LF: 0.15–0.80 Hz), used as a marker of sympathetic activity, and high-frequency spectrum (HF: 0.8–2.5 Hz), which characterizes the vagal (parasympathetic) activity of the heart. The ratio LF/HF served as a marker of the sympathetic-vagal balance in the regulation of the heart.

Experiments in anesthetized animals

At baseline, with the introductory ether anesthesia, a catheter was inserted into the left femoral vein and 1 % solution of chloralose (40–50 mg/kg) was administered. Every 30 minutes chloralose (Aldrich) injection 10 mg/kg was repeated. Ventilation was provided through tracheostomy, and hypercuronium bromide (Arduan 1 mg/h) was administered through the right femoral vein. Ventilation was performed by the device TOPO (Kent Scientific Corp) with room air at a frequency of 50–60 breaths per minute. The inspiratory air pressure was continuously monitored. BP was recorded in the left femoral artery by the sensor Siemens-Elema (model 746). Mean blood pressure was calculated by integrating the on-line BP signal with the time constant of 3 seconds. The animal was fixed on a heated surface with a thermal stabilizer, rectal temperature was maintained at the level of 37–38 °C. Left cervical sympathetic trunk was isolated from the ventral surface with the use of microscope MBS-2 (gain of 25), and it was cut at the level of the upper cervical sympathetic ganglion. Electrical nerve activity was registered by bipolar platinum electrodes with bioamplifier with a bandpass of 10–2000 Hz. The output signal was rectified and integrated on-line with the time constant of 0.1 seconds. The resulting activity was measured as μV×s. Tonic electrical nerve activity (after amplification and integration), mean and pulse blood pressure were in a digital format (Pentium-S) after the analog-to-digital conversion with a sampling rate of 100 Hz for 300 seconds. At calibration, a zero integrated activity was considered the value registered in the end of the experiment, 15–20 minutes after cardiac arrest and animal death. In each experiment, at least 4–5 recordings were registered. Each recording included calculation of the mean (300 seconds) integrated activity, average systolic BP and intersystolic interval. The obtained values were averaged for all the experimental recordings.

Table 1

THE CHANGES IN SYSTOLIC BLOOD PRESSURE, INTERSYSTOLIC INTERVAL, AND HEART RATE VARIABILITY AFTER LEFT RENAL ARTERY CLIPPING (M ± m) IN AWAKE RATS (NON-INVASIVE MEASUREMENTS ON THE CAUDAL ARTERY)

Parameters	Weeks			
	2	4	6	8
Experimental group				
BP, mm Hg	162.0 ± 6.9**	156.4 ± 6.9*	152.4 ± 8.9*	153.1 ± 7.5*
ISI, ms	151.5 ± 4.7	161.0 ± 2.5	152.6 ± 4.0	152.4 ± 2.1
LF, ms^2/Hz	30.0 ± 5.6*	22.0 ± 2.6	36.0 ± 5.0	43.9 ± 6.7*
HF, ms^2/Hz	99.1 ± 9.1	100.0 ± 3.4	106.1 ± 9.2*	108.6 ± 9.0*
LF/HF	0.31 ± 0.05*	0.23 ± 0.03	0.30 ± 0.04	0.43 ± 0.05 **
Control group				
BP, mm Hg	127.2 ± 2.6	126.4 ± 3.2	123.3 ± 2.5	129.5 ± 2.4
ISI, ms	145.7 ± 3.0	152.0 ± 3.6	165.4 ± 4.2	166.2 ± 3.4
LF, ms^2/Hz	14.3 ± 1.6	28.6 ± 5.3	36.6 ± 5.9	28.6 ± 3.3
HF, ms^2/Hz	82.6 ± 5.9	118.9 ± 10.4	144.1 ± 9.2	135.5 ± 6.3
LF/HF	0.17 ± 0.01	0.23 ± 0.03	0.25 ± 0.03	0.21 ± 0.02

Note: BP — systolic blood pressure; ISI — intersystolic interval; LF — low frequency component of heart rate variability; HF — high frequency component of heart rate variability; LF/HF — sympatho-vagal balance; ** — $p < 0.01$; * — $p < 0.05$ — significance level for the differences between the groups.

Table 2

THE CHANGES IN MEAN BLOOD PRESSURE, INTERSYSTOLIC INTERVAL, AND BIOELECTRIC ACTIVITY OF SYMPATHETIC NERVE AFTER LEFT RENAL ARTERY CLIPPING (M ± m) IN ANESTHETIZED RATS (INVASIVE MEASUREMENTS)

Parameters	Week after clipping			
	2	4	6	8
Mean BP, mm Hg	124 ± 5**	129 ± 6**	130 ± 7**	134 ± 5**
ISI, ms	141 ± 2	149 ± 4*	142 ± 2**	136 ± 2 **
EASN, mV×s	163.6 ± 17.1**	119.1 ± 11.6	151.1 ± 27.5**	157.5 ± 29.7**

Note: BP — blood pressure; ISI — intersystolic interval; EASN — electrical activity of the sympathetic nerve; ** — $p < 0.01$; * — $p < 0.05$ — significance level for the differences between the groups.

Statistical analysis

The results were processed with the use of STATISTICA 6.0 software and presented as "mean ± SEM". Comparative analysis was performed with the use of Student t-test.

Results

Before renal artery clipping, awake systolic BP was 128 ± 1 mm Hg, intersystolic interval — 152 ± 2 ms, the low-frequency component of heart rate variability (LF) — 29.4 ± 2.7 ms^2/Hz, the high frequency component of heart rate variability (RF) — 95.8 ± 3.1 ms^2/Hz, and LF/HF — 0.30 ± 0.02.

In the control group, the parameters were the following: initial systolic BP was 131 ± 1 mm Hg, the intersystolic interval — 145.0 ± 3 ms, LF — 20.8 ± 2.6 ms^2/Hz, RF — 84.4 ± 5.4 ms^2/Hz, and LF/HF — 0.23 ± 0.02. Two weeks after renal artery clipping rats from the experimental group showed elevated BP throughout the observation period (Table 1). Simultaneously LF component of heart rate variability and the sympathetic-vagal balance increased (Table 1).

In anesthetized control rats, the average BP was 95 ± 3 mm Hg, intersystolic interval — 160 ± 4 ms, integrated electrical activity of the sympathetic nerve — 93.4 ± 7.8 μV × s. Two weeks after clipping the renal artery experimental rats (n = 8) demonstrated an increase in the mean BP, heart rate, and sympathetic nerve activity. The dynamics of the sympathetic nerve electrical activity differed from the dynamics of mean BP (Table 2). Four weeks after renal artery clipping sympathetic nerve activity decreased, remaining above the reference level. Eight weeks of hypertension were associated with an increase in sympathetic nerve electrical activity up to almost maximal level (Table 2).

The association between the LF component of heart rate variability and the sympathetic nerve electrical activity had a consistent pattern. Four weeks after renal artery clipping sympathetic nerve activity was lower than 2, 6, and 8 weeks after artery clamping (Tables 1 and 2). BP level remained above baseline values in both chronic and acute experiments. At the same time, the dynamics of the parasympathetic activity remained unchanged during the whole observation period.

Discussion

At present, increased sympathetic activity is shown in most studies of renovascular hypertension. Animals with renovascular hypertension demonstrate elevated plasma norepinephrine levels [19, 20] and enhanced sympathetic nerve electrical activity [21]. It is considered one of the main mechanisms of hypertension development in both spontaneously hypertensive rats, rats kept on a high-salt diet, and animals with renal injury or ischemia. The enhancement of the sympathetic activity plays a significant role in the pathogenesis of essential hypertension [22]. The shift in the development of hypertension after renal nerve transection in spontaneously hypertensive rats and rats with renal damage confirms this hypothesis [23, 24]. Catheter radiofrequency renal ablation is a treatment approach in refractory hypertension [25–27].

Although an increase in sympathetic activity in renovascular hypertension is unquestionable, it is unclear whether the activity of preganglionic nerves is enhanced at all segments of the spinal cord, and how the change in sympathetic nerve

electrical activity correlates with BP elevation after renal artery clipping.

According to our results, renal artery clamping leads to an increase in BP associated by changes in the cervical sympathetic nerve electrical activity and enhanced sympathetic outputs to the heart, which suggests an uniform amplification of the activity of spinal preganglionic neurons in renovascular hypertension. Both heart rate variability, and bioelectrical sympathetic nerve activity decreased 4 weeks after renal artery clipping. We suggest the following assumption. The increase in angiotensin II level occurring immediately after renal artery clipping plays a significant role in the development of renovascular hypertension [20]. A gradual increase in angiotensin II level leads to the enhancement of sympathetic activity and the electrical activity of the sympathetic nerves. However, Yoshimoto et al (2010) demonstrated that bioelectric activity can be depressed in some sympathetic neurons (in case of concomitant hypertension) [28]. We can hypothesize that elevated angiotensin II levels lead to the inhibition of sympathetic neurons in the upper segments of the spinal cord (the level of heart innervation and of cervical sympathetic nerves formation) 4 weeks after renal artery clamping.

Thus, the mechanism of hypertension occurring after renal artery clipping, is rather complex, and increased vasomotor activity of spinal neurons appears to play the key role.

Conflict of interest

The authors declare no conflicts of interest.

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