Assessment of Intramural Blood Flow and Neurogenic Control in Intact and Hypertrophic Urinary Bladder with Harmonic Analysis of Bioimpedance in Rats

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 153, No. 4, pp. 422-426, April, 2012
Original article submitted February 7, 2011

High-resolution impedancometry and harmonic (Fourier) analysis of variable component of bioimpedance revealed rhythmic oscillations of urinary bladder bioimpedance at the Mayer wave, respiration, and heartbeat frequencies. The power values of the corresponding Mayer, respiratory, and cardiac peaks were calculated to assess circulation in the urinary bladder wall and its autonomic nervous control at various stages of infusion cystometry in intact rats and in the rats with preliminary formed infravesical obstruction (IVO). In intact rats, filling of the bladder with physiological saline diminished the power of the first (fundamental) cardiac peak attesting to a decrease of the blood flow in the bladder wall. Simultaneously, the power of low-frequency Mayer peak reflecting sympathetic activity increased, while the power of respiratory peak decreased supposedly reflecting abatement of the parasympathetic influences. Bladder voiding was accompanied by a decrease of Mayer peak and increase of the respiratory one. Prior to infusion cystometry, the intravesical pressure in IVO rats was elevated while the power of fundamental cardiac peak was below the control value. Filling the bladder in these rats was accompanied by further decrease of the cardiac peak reflecting still greater drop in blood supply. In control rats, voiding the bladder normalized the vesical circulation assessed by the cardiac peak, while in IVO rats this peak remained decreased. The reciprocal changes of Mayer and respiratory peaks observed during infusion cystometry in the norm were replaced by unidirectional decrease in the power of both peaks in IVO rats, which probably attest to disturbance of autonomic nervous control in the hypertrophic urinary bladder in these rats.

Key words: *impedancometry, harmonic analysis, infravesical obstruction, urinary bladder circulation, autonomic nervous control*

Urinary bladder function is determined by concordant reactions of sympathetic and parasympathetic branches of autonomic nervous system (ANS); additionally, it is pronouncedly affected by adequacy of vesical circulation [3]. Disturbances in regional circulation are considered as one of the key factors in the development of functional abnormalities shaping the symptom complex of lower urinary pathways [4,5,8,11]. The progressing chronic hypoxia of detrusor results in dystrophic alterations in leiomyocytes and in the neuroreceptor apparatus of the bladder wall [6] aggravating the developed functional disturbances.

The tools to diagnosticate the circulatory disorders in the wall of urinary bladder are rather limited.

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Both in clinics and in physiological laboratories, the state of intramural blood flow is measured only with laser Doppler flowmetry. Assessment of ANS functional activity controlling the functions of urinary bladder is limited within the data obtained in the invasive neurophysiologic or morphological studies corroborated by examination of the systemic effects (changes in AP, heart rate and its variability) [9]. To our best knowledge, design of universal method capable to simultaneously assess regional circulation and its neurogenic modulation at various levels of functional activity of the examined organ has not been reported previously.

The development of impedancometry resulted in construction of the high-resolution impedance converters capable to record the microvariations of bioimpedance, while the harmonic (Fourier) analysis of these variations revealed the spectrum peaks characterizing regional circulation and neurogenic activity [2].

This work was designed to assess the potency of high-resolution impedancometry coupled with harmonic analysis of the long-term epochs comprising hundreds cardiocycles to characterize circulation in the wall of intact and hypertrophic urinary bladder as well as its ANS control at rest and during functional activity.

MATERIALS AND METHODS

The study has been carried out on *in situ* random-bred female albino rats weighing 280-320 g (n=20). In rats narcotized with ether and thiopental sodium, the abdominal cavity was opened and the urinary bladder was isolated. Two floating nonpolarizable Ag/AgCl electrodes were attached to the bladder wall dome to minimize the mechanical artifacts of bladder motion caused by the respiratory excursions. To perform infusion cystometry with the help of infusion pump (0.09 ml/min), the bladder was catheterized via urethra with a 20G cubital catheter coupled to a pressure transducer. A custom-made hardware-software system (Biola) including an impedance converter with resolving power 250 μΩ in the range of ±4 Ω, a 4-channel digitizer PPSh-04 (Biola) and an original software [2] was employed to record simultaneously the intravesical pressure, total (basal) impedance, and alternating impedance component (Fig. 1, *a*, *b*). The rhythmical variations of vesical impedance (Fig. 1, c) were revealed with fast Fourier transform yielding the spectrograms (Fig. 1, *d*) used to calculate the power of spectral peaks.

In series I, impedancometry of filled or post-void bladder was carried out in intact (control) rats (n=10). In series II, similar measurements were performed on experimental rats with previously modeled (1 month prior to experiment) partial infravesical obstruction (IVO) caused by calibrated narrowing of the prevesical urethra by its ligation on the catheter (n=10).

The data were analyzed statistically using Statistica 6.0 software.

RESULTS

Harmonic analysis of the long-term epochs of vesical bioimpedance variations comprising hundreds cardiac cycles (Fig. 1, *c*) yielded the spectrum plots with low-frequency Mayer peak M1 (0.2 Hz), respiratory peak R1 at the respiration rate, cardiac peak C1 at the heart rate, and also the second respiratory (R2) and cardiac (C2) harmonics of smaller amplitudes (Fig. 1, *d*). According to the current views, we considered M1 and C1 peaks as reflecting, respectively, the level of regional sympathetic influences and circulation in the examined organ [2], while the nature of respiratory bioimpedance variations presented with R1 peak is a matter of discussion.

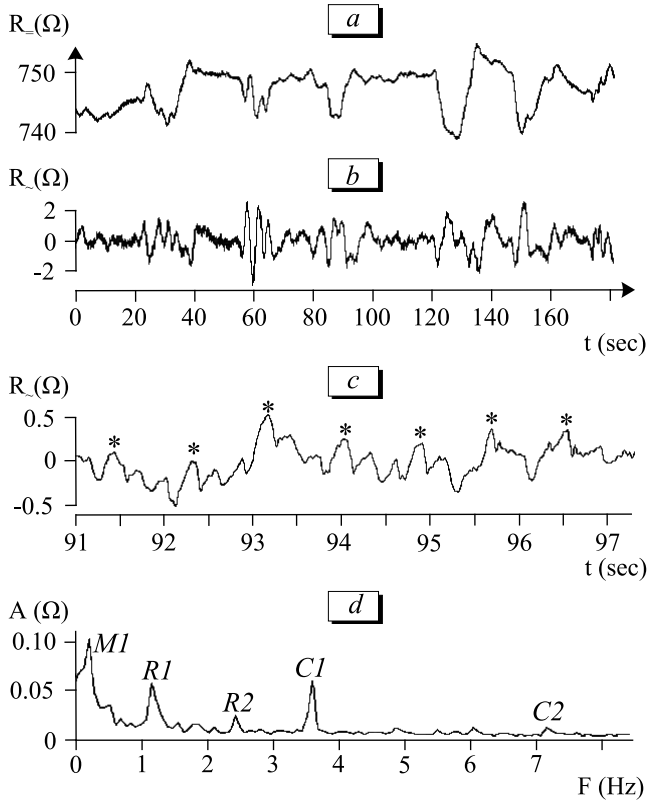

Fig. 1. Impedograms and spectrum of vesical impedance microvariations in intact rat. *a*) total (basal) vesical impedance, the large undulations reflecting spontaneous contractile activity; *b*) alternating impedance component; c) a short large-scale fragment of alternating impedance component showing the relatively large respiratory waves at the rate of about 1 Hz (marked with asterisks) and smaller cardiac waves at the rate of about 3.5 Hz; *d*) amplitude Fourier spectrum of impedance microvariations (record fragment 101-122 sec) with Mayer peak *M1* (0.2 Hz), two respiratory harmonics *R1* and *R2*, and two cardiac (pulsatile) harmonics *C1* and *C2*.

TABLE 1. Interrelation between Urinary Bladder Filling and Intramural Circulation in Intact and IVO Rats (*M*±*SEM*)

Vesical state	Detrusor pressure, cm H_2O		Cardiac peak power, 10^{-3} Ω^2	
	control	IVO	control	IVO
Initial	4.6±1.4	8.2±2.7*	0.60±0.04	0.24±0.06**
Maximum filling	28.4±1.8	53.3±2.6**	0.44±0.04	0.18±0.08**
Post-void	3.8±0.8	14.9±2.1**	0.55±0.09	0.16±0.06***

Note. *p<0.05, **p<0.01, ***p<0.001 compared to the control.

The control (intact) and IVO rats demonstrated significantly different urodynamic and circulation parameters of the urinary bladder measured in various phases of cystometry (Table 1). Prior to infusion, the basal detrusor pressure in IVO rats was higher by 77% than the control value, while the power of cardiac peak was smaller than the control one by 60%. These results agree with the data on decreased blood flow in obstructive urinary bladders obtained with alternative methods [5,12].

At the end of infusion cystometry, both groups of rats demonstrated a decreased power of cardiac peak accompanied by intravesical pressure elevation indicating impaired blood supply to the bladder wall. Similar phenomenon was observed in dogs [5]. Clinical examinations also revealed decrease of the blood flow in bladder wall by 36% during its maximum filling [7].

During cystometry in IVO rats, the intravesical pressure significantly increased and surpassed the control level nearly 2-fold. In addition, the power of cardiac peak C1 reflecting circulation in the bladder wall, decreased more pronouncedly in IVO rats (by 57% against the control value of 26%).

In control rats, the post-void vesicle pressure dropped to initial level, while in IVO rats it remained elevated in comparison with control and initial values. The power of cardiac peak increased in the control group and approximated to the initial level (attaining 92% its value), while in the experimental group, it remained decreased. Overall, the data revealed pronounced circulation disturbances in the urinary bladder of rats subjected to modeled IVO.

Simultaneous recording of intravesical pressure and bladder bioimpedance and subsequent harmonic analysis of the long-term epochs of bioimpedance variations revealed interaction between circulation and neurogenic activity in this organ during various periods of its functional activity (Fig. 2).

It is important that in intact urinary bladder, changes in power of Mayer M1 and respiratory R1 peaks during filling and voiding phases were reciprocal (Fig. 2, *a*). During the filling phase, M1 peak increased while R1 peak decreased, and the reversal changes were observed during voiding.

Up-regulation of sympathetic activity during urine accumulation in the bladder is a well-known phenomenon: this branch of ANS is considered to be responsible for relaxation of the bladder wall during filling phase. Elevation of intravesical pressure during filling phase and especially during IVO-caused urinary retention is accompanied by blood pressure elevation and simultaneous increase in activity of renal and splenic

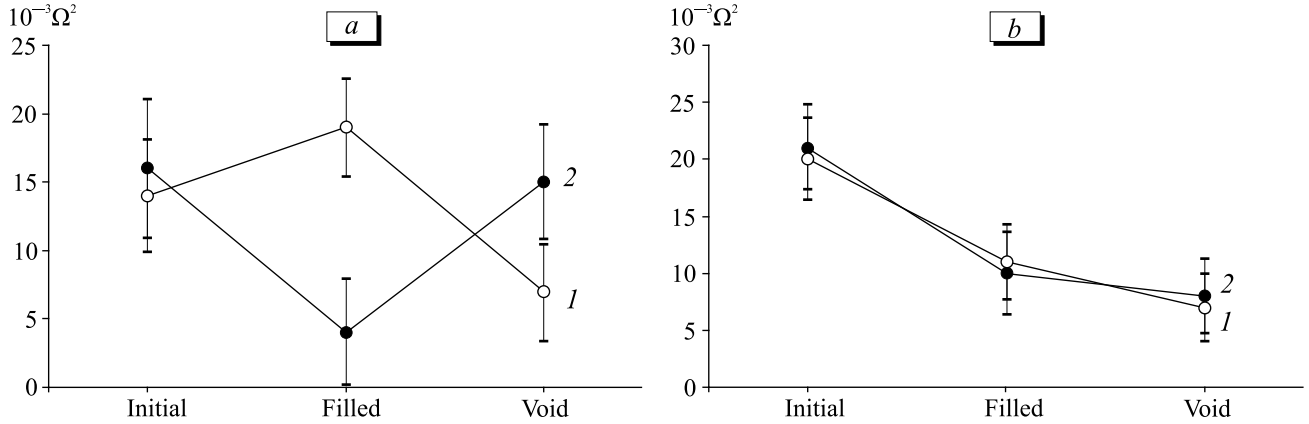

Fig. 2. The changes in the power of Mayer (*1*) and respiratory (*2*) spectrum peaks of vesical impedance variations during various phases of infusion cystometry in control (*a*) and IVO (*b*) rats.

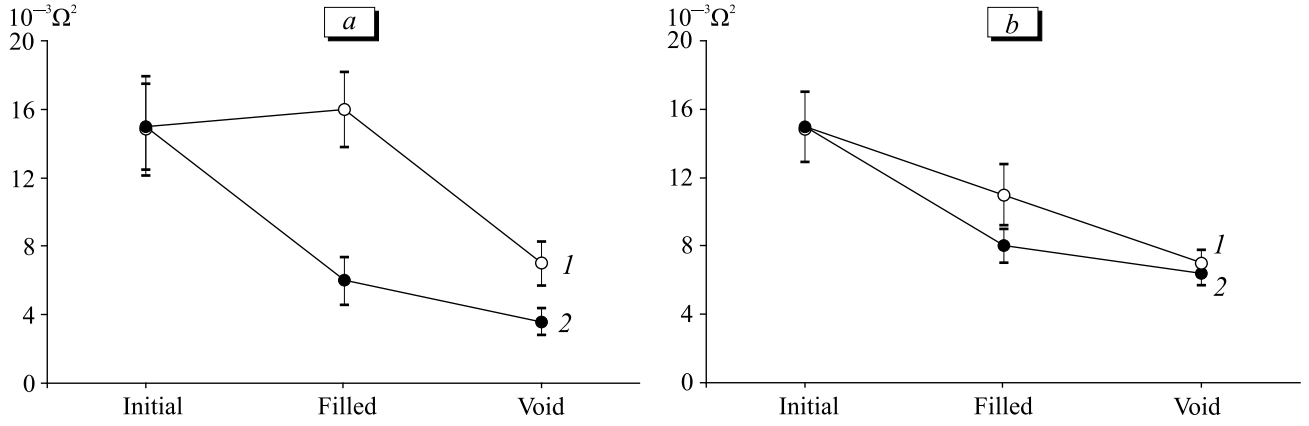

Fig. 3. Effect of *a*) intravesical (0.2 mg/liter), and *b*) intravenous (20 mg/kg) atropine on the power of Mayer (*1*) and respiratory (*2*) peaks at various cystometry phases.

nerves [15]. During urination, sympathetic activity is inhibited, while parasympathetic outflow responsible for detrusor contraction is augmented. It can be hypothesized that changes in the power of basic bioimpedance rhythms during bladder filling reflect varying levels of vesicotropic activity in both branches of ANS, which agrees with established reciprocity of sympathetic and parasympathetic influences targeted to the urinary bladder.

Increase of the respiratory peak at the end of infusion cystometry during urination can be explained by activation of parasympathetic influences. To test this hypothesis, we carried out the infusion cystometry during block of cholinoreceptors with atropine (Fig. 3). This cholinolytic drug was administered intravesically with infusion solution (0.2 mg/l) or injected intravenously (20 mg/kg). In contrast to the control tests, these experiments revealed no increase in the respiratory peak during bladder filing (Fig. 3). These data attest to a certain correlation between bioimpedance respiratory peak and activity of parasympathetic branch of ANS involved in vesical control.

Reciprocal relations between Mayer and respiratory peaks were disturbed in IVO rats. Both peaks decreased during filling and voiding phases (Fig. 2, *b*). Assuming the power ratio of Mayer to respiratory peak to reflect the corresponding ratio of sympathetic and parasympathetic activity in the regulation of the urinary bladder, we can see that in control rats, the vesical sympathetic activity overtly prevailed over the parasympathetic one, while the reverse relations were characteristic of the voiding phase; in contrast, the ratio of these activities in IVO rats was virtually constant irrespective to the state of the bladder (Table 2).

These findings attest to disturbance of the autonomic nervous regulation in hypertrophic urinary bladder in IVO rats, although the mechanism of these abnormalities and their functional consequences need further study. The reported data showed that in the hypertrophic urinary bladder, the relaxing effect of adrenal stimulation is annihilated and can be even transformed into the excitatory one [1]. During IVO, the contribution of cholinergic stimulation into vesical contractile activity is also modified. While in the normal, cholinoreceptor blockade with atropine induced complete or virtually complete suppression of detrusor contractions, in IVO this cholinolytic drug decreased contractions only by 35-75% [10,13,14]. Thus, the concerted evidence indicates an increasing role of the non-cholinergic pathways in the control of contractions of the hypertrophic detrusor.

TABLE 2. Interrelation between Mayer and Respiratory Peaks in Various Phases of Cystometry in Intact and IVO Rats (*M*±*m*)

Cystometry stage	Control	IVO
Initial	0.9±0.1	0.9±0.1
Full	4.0±0.2	0.9±0.2
Void	0.5±0.1	1.0±0.1

This work was supported by the Federal Agency for High-Technological Medical Care of the Russian Federation (grant No. 0.2740.11.0719).

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