ORIGINAL PAPERS

Adv Clin Exp Med 2013, **22**, 3, 337–346
ISSN 1899–5276
© Copyright by Wroclaw Medical University

Łukasz Dobrek[A–D], Beata Skowron[B], Agnieszka Baranowska[B], Piotr J. Thor[E, F]

Spectral Heart Rate Variability Analysis in Experimental Obstructive and Chemical Overactive Bladder Models*

Analiza widmowa zmienności rytmu serca w eksperymentalnych modelach obstrukcyjnego i chemicznego nadaktywnego pęcherza moczowego

Department of Pathophysiology, Jagiellonian University, Medical College, Cracow, Poland

A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. Overactive bladder (OAB) is a clinical entity with complex, still incompletely understood pathophysiology, involving central and peripheral autonomic nervous system (ANS) disturbances.
Objectives. The aim of the study was to estimate ANS activity using spectral analysis of heart rate variability (HRV) in two experimental overactive bladder models: chemical, evoked by cyclophosphamide treatment (COAB), and obstructive, produced by proximal partial bladder outlet obstruction (OOAB).
Material and Methods. 10 COAB rats and 10 OOAB rats with appropriate control groups were studied (40 animals total were enrolled in the study). In all studied groups, resting HRV recordings were performed. Standard spectral HRV parameters were analysed. The bladder overactivity was confirmed by urodynamic recordings and histological assessment.
Results. In COAB, all non-normalized spectral HRV parameters were diminished, while OOAB rats were mostly characterized by pronounced LF (Low Frequency) and HF (High Frequency) decrease. Normalized (nLF and nHF) parameters achieved similar values in both COAB and OOAB comparing to appropriate controls. In the analysis of percentages of the individual components in the total HRV power, the OOAB group showed almost double VLF (Very Low Frequency) percentage as compared to the control. OOAB rats also displayed the highest disproportion between VLF and both HF and LF percentages. Contrary to the OOAB, there were small differences in the percentage participation of the separate HRV components in COAB.
Conclusions. For both COAB and OOAB models, the authors demonstrated a decrease in the values of spectral HRV parameters, which may reflect ANS disturbances. Moreover, in OOAB animals, apart from total HRV power reduction, exaggerated differences between VLF percentage and the remaining components were revealed. In authors' opinion, their findings concerning VLF differences may reflect increased autonomic disturbances in the OOAB model (**Adv Clin Exp Med 2013, 22, 3, 337–346**).

Key words: overactive bladder, cyclophosphamide, bladder outlet obstruction, heart rate variability, autonomic nervous system.

Streszczenie

Wprowadzenie. Zaburzenia patofizjologiczne leżące u podstaw rozwoju nadaktywności pęcherza moczowego są złożone i wciąż nie w pełni poznane. Patogeneza tej choroby jest związana między innymi z dysfunkcją autonomicznej kontroli pęcherza.
Cel pracy. Oszacowanie aktywności autonomicznego układu nerwowego (ANS) za pomocą analizy widmowej (częstotliwościowej) zmienności rytmu serca (HRV) w dwóch doświadczalnych modelach pęcherza nadaktywnego

* This work was supported by grant number K/DSC/000953 (specific subsidy for young scientists of the Ministry of Science and Higher Education).

(OAB): chemicznego (COAB), wywołanego przez podawanie cyklofosfamidu oraz obstrukcyjnego (OOAB), związanego z częściową, podpęcherzową blokadą odpływu moczu.
Materiał i metody. Badania przeprowadzono na grupie 40 szczurów, podzielonych na dwie grupy z danym modelem OAB (COAB, OOAB) oraz dwie odpowiednie im grupy kontrolne (liczebność każdej z grup n = 10). W każdej z badanych grup przeprowadzono spoczynkowe zapisy zmienności rytmu serca, oceniając standardowe parametry widmowe. Nadaktywność pęcherza była potwierdzana badaniami urodynamicznymi oraz morfologiczną i histologiczną oceną tkanek pęcherza.
Wyniki. W grupie COAB wartości wszystkich nieznormalizowanych parametrów analizy spektralnej były zmniejszone, podczas gdy w grupie OOAB zaznaczył się głownie spadek składowej niskiej (LF) i wysokiej i (HF) częstotliwości. Znormalizowane parametry (nLF i nHF) osiągały podobne wartości w obydwu grupach z OAB i odpowiadającym im grupach kontrolnych. Oceniając procentowy udział poszczególnych składowych w mocy całkowitej widma HRV, w grupie OOAB uwidoczniono niemal dwukrotnie większy odsetek składowej bardzo niskiej częstotliwości (VLF) w porównaniu z odpowiednią grupą kontrolną. Ponadto grupę OOAB cechowała największa dysproporcja między odsetkową wartością VLF i pozostałymi składowymi widma (LF i HF). Przeciwnie, w grupie COAB, różnice między procentowym rozkładem składowych widma HRV nie były aż tak zaznaczone, w porównaniu do odpowiedniej grupy kontrolnej.
Wnioski. Zarówno w grupie COAB, jak i OOAB wykazano zmniejszenie wartości parametrów analizy widmowej HRV, co sugeruje zaburzenia autonomicznego układu nerwowego. Ponadto grupę OOAB cechowała zaznaczona dysproporcja w procentowym rozkładzie VLF i pozostałych składowych widma. Wyniki te mogą wskazywać na bardziej nasilone zaburzenia autonomicznego układu nerwowego w tym modelu OAB model (**Adv Clin Exp Med 2013, 22, 3, 337–346**).

Słowa kluczowe: pęcherz nadaktywny, cyklofosfamid, częściowa blokada podpęcherzowa, zmienność rytmu serca (HRV), autonomiczny układ nerwowy.

Overactive bladder (OAB) is a clinical entity with complex pathogenesis and symptomatology; its global prevalence approaches 11.8% (including 10.8% of men and 12.8% of women). In general, OAB affects all ages, but its occurrence increases with aging and is more common in women than in men [1].

OAB was defined in 2002 by the International Continence Society (ICS) as urinary urgency with or without urge incontinence, usually associated with frequency and nocturia, in the absence of proven bladder disorders [2]. However, the OAB description, including the diagnostic and therapeutic guidelines, has been evolving. The current OAB definition, revised in 2006, constitutes urgency with or without urgency incontinence (UI), usually with increased daytime frequency and nocturia. "Urgency" is defined as "the complaint of a sudden compelling desire to pass urine, which is difficult to defer" [3, 4]. Thus, "urgency urinary incontinence" is "the complaint of involuntary leakage accompanied by or immediately preceded by urgency"; "increased daytime frequency" is "the complaint by the patient [...] that he/she voids too often by day", while "nocturia" is defined as "the complaint that the individual has to wake at night one or more times to void" [3, 4].

The mechanisms that underlie OAB are complex and still incompletely understood. Generally, the urinary bladder plays a role in urine storage and voiding. Its functioning is regulated by the peripheral and central nervous systems. The bladder is innervated with both the sympathetic nerves (originating from the intermediolateral nuclei in the thoracolumbar region (Th10-L2) of the spinal cord) and the parasympathetic ones (arising from the sacral S2-S4 spinal parasympathetic nucleus). Detrusor contractions, which are responsible for bladder emptying, are initiated via acetylcholine (Ach) release and muscarinic receptors activation. Two main periods in bladder functioning may be distinguished: storage and emptying (voiding). Physiologically, during the storage phase, no spinal parasympathetic activity or bladder contractions occur, while an increased sympathetic discharge contributes to bladder relaxation with urethral smooth muscle and urethral and pelvic floor striated muscles keeping the outflow closed. In contrast, during voiding, the decreased sympathetic activity contributes to bladder outflow opening, which in connection with increased parasympathetic input, lead to bladder contractions and emptying. Hence, normal bladder function is dependent on the intricate interplay of neurogenic and myogenic cells; disruption of any of these may lead to urinary dysfunction [5, 6].

Two main theories, neurogenic and myogenic, have been proposed to address the pathophysiological processes. The first focuses on the central and peripheral autonomic nervous systems (ANS) disturbances affecting bladder contractility and functioning. The main neurogenic theory describes the pathophysiological problem of OAB in connection with the damage of descending inhibitory pathways arising from higher brain centers, while according to the peripheral neural theory, OAB development results from an increased sensitization of bladder afferent terminals. Regardless of the central or peripheral reason, generalized, nerve-mediated excitation of the detrusor muscle

takes place. The myogenic theory suggests that bladder over-reactivity may be associated with remodeling of the bladder wall and abnormal electrical excitation of cells resulting from excitatory stimulus spreading across a large number of coupled smooth muscle cells [5, 7]. The detailed description of both the anatomical and physiological aspects fundamental to the neural and myogenic theories of OAB development goes beyond the constraints of the present work. Basic OAB pathophysiology concepts with current management and future OAB pharmacotherapy perspectives were discussed in the authors' reviews published in the last year [8, 9].

In view of the neurogenic OAB pathophysiological theory, it is necessary to assess the autonomic nervous system. In humans, the "Ewing cardiovascular tests battery" and catecholamine level measurements evaluate ANS activity [10]. However, according to current guidelines, the heart rate variability (HRV) study is regarded to be the best non-invasive method of ANS indirect assessment for both experimental and clinical purposes. Heart rate variability phenomenon is the temporary variation of normal to normal beat intervals, observed in an ECG recording of the subject with dominant sinus rhythm, that is influenced by cardiac autonomic innervation [11, 12]. Two main types of HRV analysis may be distinguished: time domain analysis, based on mNN [ms] (main parameter) as well as other statistical derivatives, and frequency (spectral) analysis, that is based on the HRV power spectrum and its components (TP – Total Power, VLF – Very Low Frequency, LF – Low Frequency – and HF – High Frequency) assessment. Generally, the total HRV power (TP) reflects global autonomic tension, LF – sympathetic/parasympathetic tone, nLF (normalized Low Frequency) – pure sympathetic or HF, nHF (normalized High Frequency) – pure parasympathetic activity. There is no unanimous agreement concerning VLF; however, this component is believed to mirror both the various short-term physiological regulations as well as autonomic inflammatory influences [11–13].

Thus, one may ask what kind of autonomic dysfunctions are present in the two entities – hemorrhagic cystitis (during cyclophosphamide treatment) and bladder outlet obstruction, both manifested by OAB symptoms, but resulting from other pathogenic mechanisms.

The aim of this study was an autonomic activity assessment using HRV spectral analysis estimation in the two animal models of overactive bladder (OAB) – evoked by cyclophosphamide (CYP) administration (chronic chemical OAB; COAB – Chronic Overactive Bladder) and by partial urethral obstruction (bladder outlet obstruction – obstructive OAB; OOAB – Obstructive Overactive Bladder).

Material and Methods

Ethics

The study was conducted after obtaining the First Local Ethic Committee in Cracow agreement (decision No 126/2010).

Animals and Studied Groups

The authors used rats from the Pharmacy Department animal house. Firstly, after arrival at the Pathophysiology Department, the animals were kept in groups of five per cage for one week to assure them the acclimatization period to new laboratory conditions. In general, the animals were housed with the retaining of 12–12 hours day-night cycle, room temperature, standard food (Labofeed, Kcynia) and *ad libitum* water.

After the acclimatization period, the animals were randomized into studied groups. The authors carried out their experimental research in two essential groups: with COAB and OOAB models, with two suitable control populations. Therefore, four groups were studied: COAB (mean body weight 289.3±42.0 g), control group (1) (mean body weight 273.3±36.0 g), OOAB (mean body weight 243.3±13.5 g) and control group (2) (mean body weight 236.3±6.3 g). Each group consisted of ten 10-week-old female Wistar rats; thus the experiment employed 40 animals.

COAB Model

Cyclophosphamide (CYP) – an oxazaphosphorine chemotherapeutic agent, was administrated intraperitoneally (i.p.) to evoke a model of hemorrhagic cystitis (HC) with bladder overactivity. In general, taking into consideration CYP dose and route of administration, there is a possibility to induce the so-called "acute overactive bladder" (AOAB) model, or a chronic case (COAB) [14, 15]. The authors conducted their study on the COAB model caused by cyclophosphamide (Sigma-Aldrich; 75 mg/kg body weight), administered i.p. four times every third day during one week (the 1st, 3rd, 5th and 7th day). This resulted in a chronic chemical bladder inflammation [14, 15]. Two animals did not survive CYP therapy, thus, in the end, the authors studied 8 COAB rats. The progressive haematuria in all remaining COAB subjects after the last CYP dose was revealed, which

was also the next evidence for bladder inflammatory change intensification. On the 8th day, after HRV recording registration, the animals were euthanized using a pentobarbital (Morbital, Biowet, Puławy) overdose (100 mg/kg body weight).

Control Group (1)

The studied animals received saline i.p. injection four times (similarly to COAB) in similar amounts as those ones administrated in the COAB rats. HRV recordings were also performed on the 8th day of the experiment.

OOAB Model

The animals enrolled into the OOAB group underwent surgery to evoke partial bladder outlet obstruction associated with secondary bladder overactivity. The experimental method used to produce bladder outlet obstruction was described previously by several investigators [16, 17]. Briefly, rats were anesthetized i.p. with pentobarbital (brand name: Morbital, Biowet, Puławy; 35 mg/kg body weight). In the surgical procedure, the authors exposed the bladder and proximal urethra through an abdominal midline incision and placed a 1 mm diameter stainless steel rod to the proximal urethra to tie a 4/0 silk ligature around the inserted rod. After experimental urethral narrowing, the catheter was removed, leaving the urethra partially occluded. The abdominal wall was sutured and the animals were allowed to recover. During the first three days of the recovery period, two antibiotics (neomycin – Neomycin spray, oxytetracycline – Oxycort spray) were administrated to the surgery wound to prevent inflammatory complications. At 2 weeks after surgery, HRV recordings were collected.

Control Group (2)

10 rats underwent sham surgery in which the authors also exposed the bladder and catheterized the proximal urethra but without its ligation. The post-surgery protocol was similar to the OOAB animals. Consistently, HRV studies were performed 2 weeks after the sham procedure, similarly to the OOAB animals.

HRV Studies

20 minute ECG recordings were performed in resting conditions under urethane anesthesia (1200 mg/kg body weight; Sigma-Aldrich) in the studied animals. Urethane was chosen based on literature reports suggesting its proportional (up to the applied dose) influence on both sympathetic and parasympathetic tonic activity, and a relatively small impact on cardiac reflexes [18, 19]. After extrasinusal extopics elimination, the remaining sinus ECG signal was subjected to HRV analysis to obtain standard spectral (VLF, LF, HF and TP – all expressed in [ms*ms] and normalized nLF and nHF, calculated in normalized units [n.u.]) parameters. The authors set the range for the respective spectral components at: $0.18<VLF<0.28<LF<0.78<HF<3$; and admitted commonly accepted interpretation criteria, as mentioned in the introduction. The initial results were calculated as values ± SD.

Bladder Overactivity Verification

In the last step, after HRV registration, 45 minute urodynamic recordings were conducted to confirm bladder overactivity in all the studied groups.

The authors also performed the urodynamic recordings using urethane because this anesthetic agent is regarded to have a relatively small potential for impairing urinary bladder motility, as mentioned previously [18, 19]. In the still anaesthetized rat, the abdomen was opened and the bladder was exposed using similar surgery as that applied in the OOAB and control (2) groups. A urodynamic polyethylene catheter (external diameter 0.97 mm/internal diameter 0.58 mm, BALT Poland), was inserted into the bladder dome through a small incision and fixed in place with 4-0 silk ligature. The catheter was connected to a pressure transducer that is a part of the analysis hardware (Power Lab; Chart 5 Pro v.5.4.2, AD Instruments). After bladder catheterization and system calibration, a 15 minute stabilization period was allowed. Following the initial procedures, the baseline recordings were registered during continuous saline infusion at a rate of 0.046 ml/min for 30 minutes (using an injection pump Unipan 340A). During the urodynamic studies, as well as the HRV ones, the studied animals were put under a heating lamp to avoid a body temperature fall through the uncovered abdominal layers that could negatively influence bladder activity and autonomic tension. Based on the obtained urodynamic recordings, the authors calculated standard urodynamic parameters (peak number – PN, peak number per minute – PNM, intercontraction interval – ICI [s] and micturition voiding pressure – MVP [cm H_2O]) to ascertain bladder contractility.

The examples of registered urodynamic figures, obtained in both control groups 1 and 2 (Fig. 1), as well as in the COAB (Fig. 2) and OOAB (Fig. 3) animals are given below.

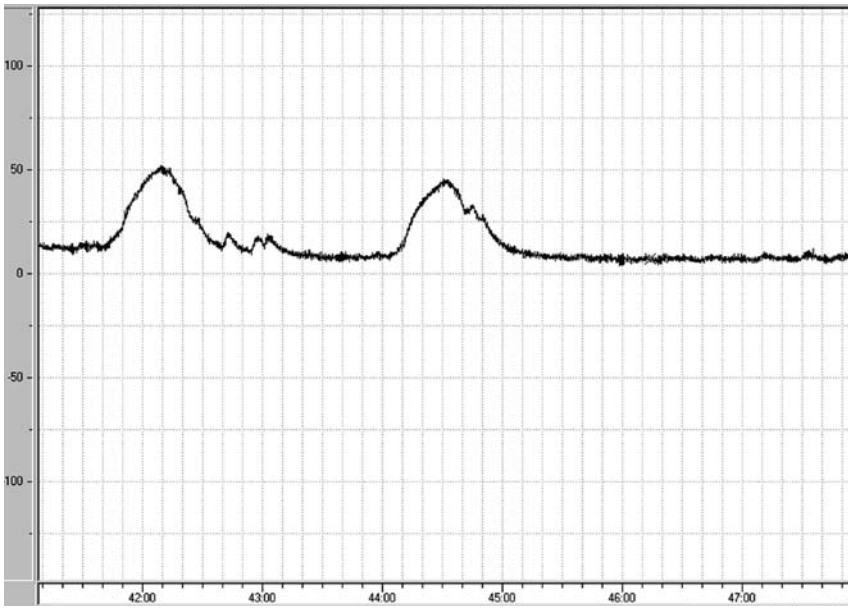

Fig. 1. An example of the urodynamic recordings obtained in control rats

Ryc. 1. Fragment zapisu urodynamicznego rejestrowanego w grupie kontrolnej

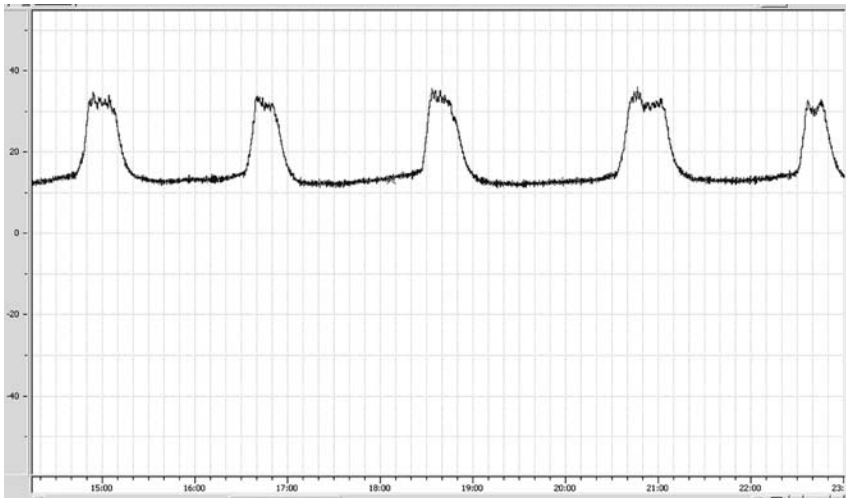

Fig. 2. An example of the urodynamic recordings obtained in COAB rats

Ryc. 2. Fragment zapisu urodynamicznego rejestrowanego w grupie COAB

The authors checked that both the COAB and BOO (Bladder Outlet Obstruction) animals displayed urodynamic OAB findings by performing a visual urodynamic records inspection as well as calculating the values of urodynamic parameters. In the case of these groups, the authors observed increased global bladder contractility (manifested in PN together with PNM increase and ICI and MVP decrease, as compared to the control).

The control rats (sham-operated or treated) displayed normal bladder contractility without urodynamic features of its overactivity. The authors have concluded that this finding may also be regarded as further indirect evidence of bladder disturbances recorded in BOO animals.

Statistical Analysis

The authors performed a statistical estimation of the obtained results in paired studied groups (COAB vs. control 1 and OOAB vs. control 2), after expressing them in the natural logarithmic values, using a parametric Fischer-Snedecor test with $\alpha = 0.05$. The necessity of the HRV spectral parameter values' expression in the form of their natural logarithms was the consequence of the lack of their normal distribution. The authors verified the null (H0) hypothesis of equality of analyzed parameter variations in the two studied populations versus the alternative H1 hypothesis of their inequality (thus, their statistically significant differences).

Urinary Bladder Assessment

The bladders were collected in both COAB and OOAB rats, as well as in the controls, after urodynamic recordings and pentobarbital overdose (100 mg/kg body weight). In the OOAB subjects, before bladder excision, the authors carefully removed the urethral ligature. In both studied

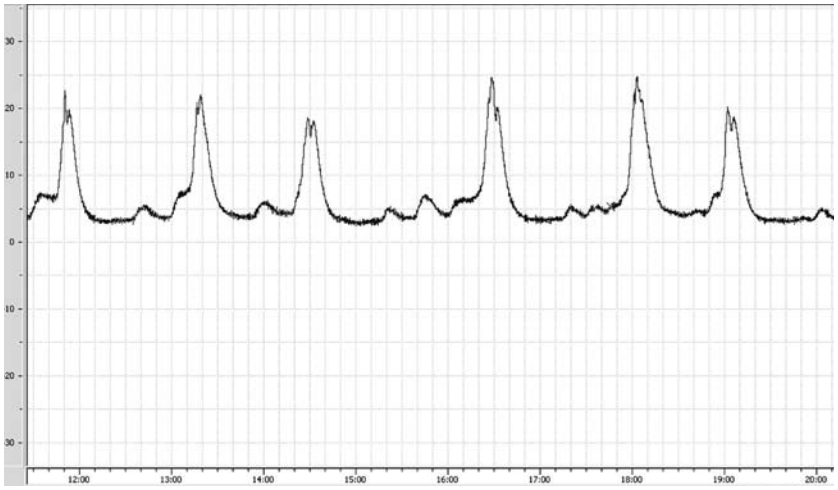

Fig. 3. An example of the urodynamic recordings obtained in OOAB rats

Ryc. 3. Fragment zapisu urodynamicznego rejestrowanego w grupie OOAB

Table 1. HRV frequency (spectral) domain analysis parameters

Tabela 1. Parametry analizy widmowej (spektralnej) HRV

HRV spectral parameter (Wskaźnik analizy widmowej HRV)	Groups (Grupy)		Statistical analysis (Analiza statystyczna)	Groups (Grupy)		Statistical analysis (Analiza statystyczna)
	COAB	control (1)		OOAB	control (2)	
ln TP	0.24 ± 1.10	1.65 ± 1.09	p = 0.002	2.06 ± 1.77	3.46 ± 0.59	p = 0.027
ln VLF	0.03 ± 1.19	1.44 ± 1.22	p = 0.003	1.89 ± 1.84	2.56 ± 1.16	p = 0.208
ln LF	–3.24 ± 1.25	–1.57 ± 1.17	p = 0.001	–1.11 ± 2.00	1.60 ± 0.47	p = 0.002
ln HF	–2.02 ± 1.21	–0.77 ± 0.82	p = 0.002	–0.88 ± 1.80	2.18 ± 0.86	p = 0.001
LF/HF	0.51 ± 0.55	0.67 ± 0.64	p = 0.255	1.06 ± 0.75	0.70 ± 0.63	p = 0.183
nLF [n.u.]	27.54 ± 19.61	33.76 ± 19.50	p = 0.216	45.2 ± 19.67	36.61 ± 16.04	p = 0.199
nHF [n.u.]	72.46 ± 19.62	66.25 ± 19.50	p = 0.216	54.8 ± 16.72	63.39 ± 16.04	p = 0.198

groups, the authors revealed gross inflammatory changes (the bladders were swollen, red, and in most cases covered by abundant serosal petechial suffusions). In a microscopic bladder assessment, leukocyte infiltration was demonstrated in both OOAB and COAB groups, however, the observed histological inflammatory lesions were more pronounced in rats with HC development due to CYP treatment. The control rats displayed no morphological or histological bladder abnormalities.

Results

The statistical estimation of spectral HRV parameters (expressed as ln values) showed intergroup differences (control 1 vs. COAB and control 2 vs. OOAB) referring to all non-normalized spectral parameters.

With regards to COAB and control 1 group, it should be noted that all non-normalized spectral HRV parameters were lower in COAB rats and the differences were statistically significant. In OOAB, a pronounced decrease in LF and HF values was revealed when compared to control 2 group, whereas VLF reached almost the same value in both analyzed populations. OOAB rats were also characterized by a low TP value. Contrariwise to the results described above, the normalized (nLF and nHF) spectral parameters achieved in all studied groups (COAB and OOAB) were similar as compared to the suitable controls and did not possess statistical significance.

The results referenced above are given in Table 1.

Moreover, intergroup differences (control 1, COAB, control 2, OOAB) of the non-normalized HRV parameters (VLF, LF, HF) were also calculated as an expression of their percentage participation related to the total power in each group. When compared in the appropriate pairs (COAB vs. control 1 and OOAB vs. control 2), more pro-

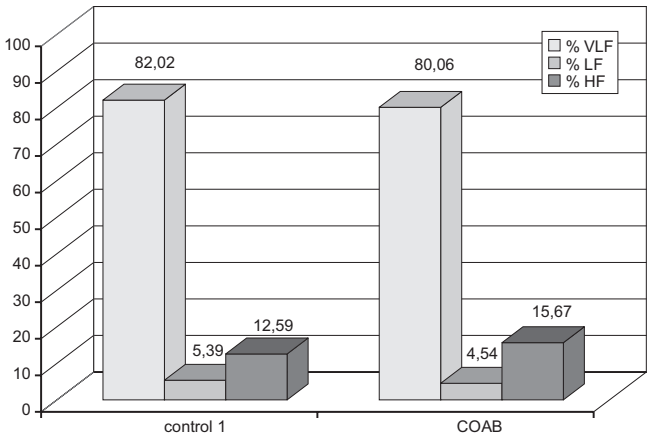

Fig. 4. Percentage participation in total power of particular spectral components in control and COAB rats

Ryc. 4. Udział procentowy poszczególnych składowych widma w grupie kontrolnej i COAB

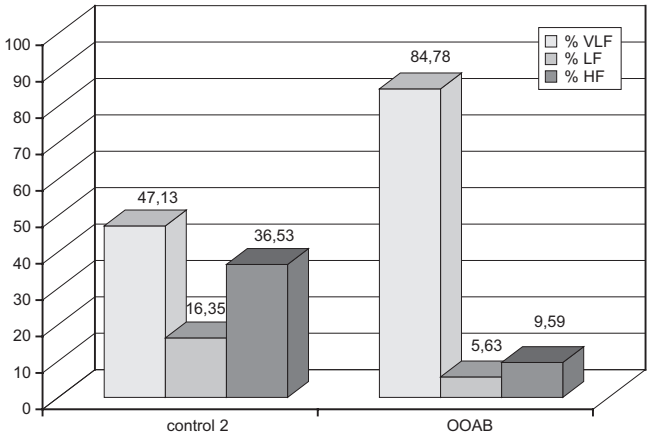

Fig. 5. Percentage participation in total power of particular spectral components in control and OOAB rats

Ryc. 6. Udział procentowy poszczególnych składowych widma w grupie kontrolnej i OOAB

nounced differences were revealed for the animals from OOAB/control 2 than from COAB/control 1. The OOAB group showed almost double the VLF percentage compared to control 2, while LF% was almost three times, and HF% – almost four times lower. OOAB rats also displayed the highest disproportion between VLF and HF percentages among all the analyzed groups. Contrary to the OOAB and control 2 rats, the differences in percentage participation of the separate HRV components in COAB and control 1 group were very small. In these populations, similar values of HRV power spectrum percentage distribution were observed. The results described above are presented in Figures 4 and 5 below.

Discussion

The main conclusion of this study was the demonstration of autonomic abnormalities in both the CYP-induced hemorrhagic cystitis (COAB) and bladder outlet obstruction (OOAB). Both COAB and OOAB groups were characterized by diminished global ANS tension and lower values of the non-normalized HRV components. These differences were especially pronounced for LF and HF. However, in both groups, normalized LF and HF comparing to appropriate controls did not differ significantly. Thus, the VLF differences seem to possess a particular significance. The VLF relations to the remaining HRV components were more visible in the intergroup comparison when the percentages of individual HRV components in each group were calculated (Figures 1 and 2). In OOAB/control 2 groups, the disproportion between %VLF/%LF/%HF was clearly emphasized, while in COAB/control 1 they were not so distinct.

The TP and all non-normalized HRV parameters decrease in both OAB rats may be a consequence of autonomic damage caused by either chemical or mechanical chronic stimuli. According to the HRV components interpretation, it seems that autonomic disturbances observed in both COAB and OOAB rats affect both the sympathetic and parasympathetic branch. A common agreement exists that efferent vagal activity is a major contributor of the HF component. Many experimental and clinical studies of autonomic maneuvers, such as electrical vagal stimulation, vagotomy, respiratory sinus arrhythmia, or mus-

carinic receptor blockage/excitation have produced pronounced changes in HF tension. The interpretation of the origin of the LF component is more controversial and ambiguous. LF is considered to be either a marker of pure sympathetic modulation (especially when expressed in normalized units – nLF increase is observed during tilt or mental or physical stress which activates the sympathetic branch) or a parameter reflecting both the sympathetic and parasympathetic influences. Moreover, during sympathetic overexcitation, LF power was even found to be diminished, reflecting the decreased responsiveness of the sinus node to the neural inputs [11, 20].

A reduction in HRV has mostly been observed in cardiac diseases. Patients with myocardial infarction, following cardiac transplantation or suffering from chronic heart failure, are characterized by decreased heart rate variability. Similarly, patients with diabetes also present reduced HRV and this finding is regarded to be a marker for the development of autonomic neuropathy [11, 20].

In the urology field, studies focused on ANS assessment and HRV are rare. In a Choi et al. study [21], lower spectral parameters in female patients with idiopathic OAB were found, thus suggesting impaired autonomic functioning in this population. In one of the latest published works, Ben-Dror et al. [22] also demonstrated a reduced sympathetic tone (reflected in lower LF values) during bladder filling in women with idiopathic OAB. Moreover, they revealed that in women with OAB, sympathetic activity decreased before maximal bladder capacity was reached. Because continual increase in sympathetic activity during bladder filling is necessary to enable the bladder to expand without detrusor contractions, the observed sympathetic dysfunction may result in sensation of urgency [22].

In another study, Juszczak et al. [23], using autonomic Ewing's battery tests, revealed resting sympathetic overstimulation in benign prostatic hyperplasia (BPH) patients with concurrent bladder overactivity symptoms. Moreover, diminished suspected parasympathetic response to vagal stimulation during deep breathing tests was demonstrated. Taking into consideration the results obtained, they concluded that these patients developed mixed autonomic disturbances [23]. Other investigators confirmed autonomic neuropathy in BPH patients. Both Mc Vary et al. [24] and Meigs et al. [25] found that patients with BPH are more likely to have sympathetic overactivation. They also revealed an indisputable dependence between BPH development and sympathetic overactivity. Thus, according to these authors, ANS disturbances may have a causal meaning in BPH development and progression.

It is difficult to uncritically and simultaneously compare results obtained from clinical and experimental results. There are essential methodology differences (e.g. Ben-Dror [22] – HRV estimating throughout monitored bladder filling; the present experiment – HRV assessment independently on bladder phase) and also the important limitations of animal modeling. First of all, OAB is a symptom-based condition in which the conscious perception of urgency is central to the diagnosis. There is no way of knowing certainly whether an animal is experiencing urgency, even if pseudo-affective changes in behavior may suggest it. Moreover, none of the animal models can reproduce all the facets of human conditions. Thus, it must be emphasized that an OAB animal model is a tool rather than a replica and is usually only relevant to the complex human condition with regards to resolving a particular experimental hypothesis. However, it is widely accepted that studies using muscle strips or animal OAB models provide a crucial link to the clinical context, enabling both a more detailed pathophysiological description and discovering more effective treatment [26].

Most animal models used to study OAB are induced models, meaning that a relevant pathophysiological challenge is experimentally applied to a healthy animal. One of the peripheral models, chronic chemical hemorrhagic cystitis (HC), results from direct damage to the bladder evoked by chronic or single cyclophosphamide (CYP) administration. This model, first proposed by Cox [27], reflects bladder damage with its overactivity in the course of oxazaphosphorine therapy and is frequently used, often with minor modifications, by numerous researchers in the OAB field. The other OAB experimental model, reflecting bladder outlet obstruction (BOO), produces storage symptoms that often persist even after the obstruction is surgically corrected. Disturbances similar to those observed in human BOO are relatively straightforward to reproduce in experimental conditions by ligating the urethra to produce partial obstruction. This experimental approach also reflects urethral obstruction due to benign prostatic hyperplasia (BPH) in men, often associated with OAB symptoms [26, 28]. Thus, much of the current knowledge from this field has come from experimental studies and not only from clinical ones.

However, despite the restrictions mentioned above, the authors again revealed the decrease in the main HRV spectral parameters in both OAB features – chemically and mechanically induced. However, in COAB, the global ANS activity decrease was accompanied by maintaining the mutual proportion between HRV individual components as compared to the appropriate control

group (Fig. 1). Contrary to this, in the OOAB animals, apart from total power reduction, there were differences between VLF percentage and the remaining components with regards to VLF increase in total ANS activity. Hence, in the authors' opinion, the VLF apparent, relative increase in OOAB may constitute the key element, depending on the causative character (mechanical or chemical) of the OAB etiological agent. However, it is not possible to interchangeably elucidate whether augmented sympathetic or parasympathetic tension contributes to the revealed VLF changes, because the mechanisms responsible for VLF development in the HRV spectrum remain unknown and require further clarifying.

As was already mentioned in the introduction, VLF is regarded as reflecting neuroendocrine rhythms, thermoregulatory actions, renin-angiotensin system activity, and hemodynamic feedback delays. Regardless of the only partly defined VLF origin mechanisms, there is great interest in this component with regards to humans. The slow rhythms reflected in the HRV spectrum in very low – VLF and even ultra-low frequencies – ULF ranges are said to be predictors of severe cardiac disorders, including cardiac death [11]. Thus, despite the fact that an unambiguous VLF meaning is still not clearly determined, most HRV guidelines consider this spectral component as a determinant of sympathetic tension. In this view, the authors' findings regarding VLF in the OOAB group may be due to sympathetic overactivity associated with simultaneous parasympathetic withdrawal. On the other hand, there are also premises to regard VLF as the reflection of parasympathetic excitation. According to Taylor et al. [29], VLF mainly results from the parasympathetic outflow, not the sympathetic. These authors demonstrated that atropine administration led to almost complete VLF power abolishment and other spectral components powers reduction, suggesting that the VLF band is driven by parasympathetic tension. Silva Soares et al. [30] revealed in their study that stimulation with pyridostigmine (a reversible cholinesterase inhibitor) produced a strong increase in VLF [30]. Therefore, contrary to the clinical observations in cardiology suggesting a strong link between VLF power and sympathetic-mediated severe cardiac disturbances, it seems that VLF oscillations may also derive from the parasympathetic mechanisms. Taking into account such a point of the view, the authors' VLF findings may also reflect parasympathetic predominance, contributing to the exaggerated but ineffective bladder motor activity in bladder outlet obstruction and resulting in overactive bladder symptoms. Chemical bladder damage caused by CYP and its metabolites could be reflected to a larger degree in the range beyond very low frequency.

The authors revealed autonomic nervous system disturbances in both CYP-induced hemorrhagic cystitis (COAB) and bladder outlet obstruction (OOAB).

Both COAB and OOAB groups were characterized by diminished global ANS tension and lower values of LF and HF values. However, in both groups, normalized LF and HF did not differ significantly when compared to appropriate control subjects. VLF percentage, calculated as an expression of its participation related to the appropriate total power was the highest in OOAB. Contrary to these findings, COAB rats displayed very small differences in percentage participation of the separate HRV components. Thus, the VLF differences seem to possess a particular significance in OAB associated with bladder outlet obstruction.

The authors realize that the conclusion on the differential role of VLF in OAB may be premature and too far-reaching. However, in their opinion, this finding seems to be very interesting and worth detailed exploration, being a promising research direction for further theoretical and clinical studies.

References

[1] **Irwin DE, Milsom I, Hunskaar S, Reilly K, Kopp Z, Herschorn S, Coyne K, Kelleher C, Hampel C, Artibani W, Abrams P:** Population-based survey of urinary incontinence, overactive bladder and other lower urinary tract symptoms in five countries: results of the EPIC study. Eur Urol 2006, 50, 1306–1315.

[2] **Abrams P, Cardozo L, Fall M, Griffiths D, Rosier P, Ulmstein U, van Kerrebroeck P, Victor A, Wein A:** The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. Neurourol Urodyn 2002, 21, 167–178.

[3] **Abrams P, Artibani W, Cardozo L, Dmochowski R, van Kerrebroeck P, Sand P:** Reviewing the ICS 2002 terminology report: The ongoing debate. Neurourol Urodyn 2006, 25, 293.

[4] **Hashim H, Abrams P:** Overactive bladder: an update. Curr Opin Urol 2007, 17, 231–236.

[5] **Andersson KE, Pehrson R:** CNS involvement in overactive bladder. Drugs 2003, 63, 2595–2611.

[6] **Abrams P, Andersson KE:** Muscarinic receptor antagonists for overactive bladder. BJU Int 2007, 100, 987–1006.

[7] **Chu FM, Dmochowski R:** Pathophysiology of overactive bladder. Am J Med 2006, 119(3 Suppl. 1), 3S–8S.

[8] **Dobrek Ł, Juszczak K, Wyczółkowski M, Thor P:** Overactive bladder (OAB) – current definition and basic pathophysiological concepts. Adv Clin Exp Med 2011, 20, 119–129.

[9] **Dobrek Ł, Juszczak K, Wyczółkowski M, Thor P:** Current management and future perspectives of overactive bladder (OAB) pharmacotherapy. Acta Pol Pharm Drug Res 2011, 68, 807–821.
[10] **Zygmunt A, Stanczyk J:** Methods of evaluation of autonomic nervous system function. Arch Med Sci 2010, 6, 11–18.
[11] **Malik M, Bigger JT, Camm AJ:** Guidelines. Heart rate variability. Standards of measurements, physiological interpretations and clinical use. Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology. Eur Heart J 1996, 17, 354–381.
[12] **Rajendra Acharya U, Joseph KP, Kannathal N, Lim CM, Suri JS:** Heart rate variability: a review. Med Bio Eng Comput 2006, 44, 1031–1051.
[13] **De Jong MMJ, Randall DC:** Heart rate variability analysis in the assessment of autonomic function in heart failure. J Cardiovasc Nurs 2005, 20, 186–195.
[14] **Dinis P, Churrua A, Avelino A, Yaqoob M, Bevan S, Nagy I, Cruz F:** Anandamide-evoked activation of vanilloid receptor 1 contributes to the development of bladder hyperreflexia and nociceptive transmission to spinal dorsal horn neurons in cystitis. J Neurosci 2004, 24, 11253–11263.
[15] **Chopra B, Barrick SR, Meyers S, Beckel JM, Zeidel ML, Ford APDW, de Groat WC, Birder LA:** Expression and function of bradykinin B1 and B2 receptors in normal and inflamed rat urinary bladder urothelium. J Physiol 2005, 562(Pt 3), 859–871.
[16] **Calvert RC, Thompson SC, Khan MA, Mikhailidis DP, Morgan RJ, Burnstock G:** Alternations in cholinergic and purynergic signalling in a model of the obstructed bladder. J Urol 2001, 166, 1530–1533.
[17] **Das AK, Leggett RE, Whitbeck C, Eagen G, Levin RM:** Effect of doxazosin on rat urinary bladder function after partial outlet obstruction. Neurol Urodyn 2002, 21, 160–166.
[18] **Maggi CA, Meli A:** Suitability of urethane anesthesia for physiopharmacological investigations in various systems. Part 1: general considerations. Experientia 1986, 42, 109–114.
[19] **Maggi CA, Meli A:** Suitability of urethane anesthesia for physiopharmacological investigations in various systems. Part 2: cardiovascular system. Experientia 1986, 42, 292–297.
[20] **Sztajzel J:** Heart rate variability: a noninvasive electrocardiographic method to measure the autonomic nervous system. Swiss Med Wkly 2004, 134, 514–522.
[21] **Choi JB, Cha MH, Seo KS, Ahn BC, Kim BT, KIm YS:** Analysis of heart rate variability in patients with an overactive bladder. Continence 2005, 1, 9–12.
[22] **Ben-Dror I, Weissman A, Leurer MK, Eldor-Itskovitz J, Lowenstein L:** Alternations of heart rate variability in women with overactive bladder syndrome. Int Urogynecol J 2012, 23, 1081–1086.
[23] **Juszczak K, Mazur M, Wyczółkowski M, Filipek M, Thor PJ:** Autonomic nervous system activity in patients with lower urinary tract symptoms secondary to benign prostatic hyperplasia estimated by heart rate variability. Open Urol Nephrol J 2008, 1, 44–49.
[24] **Mc Vary KT, Rademaker A, Lloyd GL, Gann P:** Autonomic nervous system overactivity in men with lower urinary tract symptoms secondary to benign prostatic hyperplasia. J Urol 2005, 174(4 Pt1), 1327–1433.
[25] **Meigs JB, Mohr B, Barry MJ, Mc Naughton CM, Mc Kinlay JB:** Risk factors for clinical benign prostatic hyperplasia in a community-based population of healthy aging men. J Clin Epidemiol 2001, 54, 935–944.
[26] **Parsons BA, Drake MJ:** Animal models in overactive bladder research. In: Handbook of Experimental Pharmacology 202. Eds.: Andersson KE, Michel MC, Berlin Heidelberg: Springer-Verlag 2011, 15–43.
[27] **Cox PJ:** Cyclophosphamide cystitis – identification of acroleine as the causative agent. Biochem Pharmacol 1979, 28, 2045–2049.
[28] **Mc Murray G, Casey JH, Naylor AM:** Animal models in urological disease and sexual dysfunction. Br J Pharmacol 2006, 147(Suppl. 2), S62–S79.
[29] **Taylor JA, Carr DL, Myers CW, Eckberg DL:** Mechanisms underlying very low frequency RR-interval oscillations in humans. Circulation 1998, 98, 547–555.
[30] **Silva Soares P, da Nobrega ACL, Ushizima MR, Irigoyen MCC:** Cholinergic stimulation with piridostigmine increases heart rate variability and baroreflex sensivity in rats. Auton Neurosci 2004, 113, 24–31.

Address for correspondence:

Łukasz Dobrek
Department of Pathophysiology
Jagiellonian University, Medical College
Czysta Str. 18
31-121 Cracow
Poland
Tel./fax: 12 632 90 56
E-mail: lukaszd@mp.pl

Conflict of interest: None declared

Received: 1.10.2012
Revised: 14.01.2013
Accepted: 13.06.2013