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## Heart rate variability after BRL37344, a beta-3 agonist, in experimental bladder outlet obstruction

Analiza zmienności rytmu serca (HRV) po podaniu BRL37344, agonisty receptora beta-3 adrenergicznego, w doświadczalnym modelu blokady podpęcherzowej

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### Summary

**Introduction:**

Bladder overactivity symptoms accompany benign prostatic hyperplasia (BPH) syndrome. The autonomic nervous system (ANS) disturbances may be involved in bladder dysfunction. An ameliorating effect on bladder overactivity is being assigned to the currently investigated  $\beta$ -3 adrenoreceptor agonists. However, little is known about the influence of  $\beta$ -3 agonists on ANS activity. The aim of our study was to estimate ANS activity using heart rate variability (HRV) in experimental model of bladder outlet obstruction (BOO), reflecting human BPH.

**Material/Methods:**

30 female rats, divided into control, non-treated BOO (LLBOO), and  $\beta$ -3 agonist (BRL37344) BOO treated (LLBOO+ $\beta$ 3 agonist) were studied. BOO was evoked by 5-week long partial proximal urethra ligation. Next, 20-minute resting HRV recordings were performed in each of the studied groups following i.p. administration of the vehicle (LLBOO) or BRL37344 (LLBOO+ $\beta$ 3 agonist).

**Results:**

LLBOO rats were characterized by diminished NN range, SDNN, and rMSSD in time-domain analysis. Similarly, TP and non-normalized spectral HRV parameters were also decreased. Contrary to these findings, normalized spectral parameters were lower (nLF) and higher (nHF). The animals treated with BRL37344 demonstrated no significant differences in time-domain HRV parameters. In spectral analysis, a decrease in LF and HF, together with a fall in TP, was found. Moreover, both nLF and nHF reached almost the same values in control and  $\beta$ -3 agonist treated rats.

**Discussion:**

Our data indicates that BRL37344 is an agent abolishing the autonomic imbalance in experimental BOO, which may contribute to relieving the symptoms of bladder overactivity in  $\beta$ -3 agonists treated participants.

**Keywords:**

**overactive bladder (OAB) • bladder outlet obstruction (BOO) • autonomic nervous system (ANS) • heart rate variability (HRV) •  $\beta$ -3 agonist**

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**Abbreviations:** **ANS** – Autonomic Nervous System, **BOO** – Bladder Outlet Obstruction, **BPH** – Benign Prostatic Hyperplasia, **BRL37344** – a  $\beta$ -3 agonist used in our study, **BWW** – Bladder Wet Weight, **HF** – High Frequency (HRV spectral component), **HRV** – Heart Rate Variability, **ICS** – International Continence Society, **LF** – Low Frequency (HRV spectral component), **LLBOO** – Long-Lasting Bladder Outlet Obstruction, **LUT** – Lower Urinary Tract, **nHF** – normalized High Frequency (HRV spectral component), **nLF** – normalized Low Frequency (HRV spectral component), **NO** – Nitric Oxide, **OAB** – Overactive Bladder, **rMSSD** – the square root of the mean squared difference of successive NN intervals, **SDNN** – Standard Deviation of all normal-normal (NN) intervals, **VLF** – Very Low Frequency (HRV spectral component).

## INTRODUCTION

Overactive bladder (OAB) is a clinical condition currently classified by the International Continence Society (ICS) as a symptom syndrome suggestive of lower urinary tract dysfunction. It is defined as denoting urgency with (“wet” OAB) or without (“dry” OAB) urge incontinence, usually with increased daytime frequency and nocturia, and in the absence of local or metabolic factors to explain these symptoms [38,40]. Notably, the current description of OAB is not only based on the urodynamic findings. Though the notion of “overactive detrusor” has appeared in previous ICS publications, and sometimes was used interchangeably with OAB, it was never the intention of the ICS committee, however, to apply OAB definition only to the urodynamic status. Originally, overactive detrusor was an urodynamic-based term, reflecting abnormal, involuntary detrusor contractions (which may be spontaneous or provoked) during filling cystometry. Overactive detrusor may be considered in terms of detrusor hyperreflexia (caused by neurologic disturbances) or unstable detrusor (caused by non-neurogenic factors). The latter was often described as “idiopathic detrusor instability” [38]. Thus, the multitude of employed qualifications motivated ICS to standardize the overactive bladder terminology and publish diagnostic guidelines. The currently endorsed definition of OAB is based on patient’s subjective perception of lower urinary tract dysfunction and assigns a lesser significance to cystometry [38]. Urgency, a sensation associated with bladder filling, is a key OAB symptom. It is considered to be difficult to describe to those who have not experienced it; however, it may be described as a sudden, and difficult to defer, compelling desire to pass urine. This complaint leads to the remaining urinary symptoms – diminished intervoiding intervals (increased urination frequency; pollakiuria), nocturia, incontinence and reduced urinary volume [8]. Increased daytime frequency is the complaint of voiding too often during the day. Nocturia is the necessity to get up one or more times during the night to void. Urge incontinence (in the so called “wet” OAB) is characterized by a strong desire to void together with an involuntary urine leakage [38].

There are difficulties in evoking OAB for the purposes of medical experiments because it is hard to unequivocally ascertain whether an animal is experiencing urgency.

Therefore, as it is not technically possible to create an ideal OAB model, surrogate models are employed, including bladder outlet obstruction (BOO). This model is characterized by bladder overactivity initially conditioned by anatomical abnormalities of the lower urinary tract and not by primary neurogenic and/or myogenic disturbances of the bladder [32].

OAB is a relatively common medical condition, affecting 12–18% of the population, and known to increase with age in both genders; although, women seem to be affected to a larger extent [12]. In USA, it is estimated that one in 11 adults suffers from OAB, and a total of 17 million adults are affected [6]. In Europe, the Milsom et al. [30] study revealed that 16.6% of all respondents (15.6% of men and 17.4% of women) have reported symptoms suggestive of OAB. However, it must be emphasized that OAB prevalence depends on the design of the epidemiological study; mostly, on the OAB inclusion/exclusion criteria. The overall consensus is that urgency and frequency constitute the main complaints, while urge incontinence is displayed by only a third of the patients. However, all OAB symptoms are taxing for the patients and have a negative impact on the quality of life [15].

The pathophysiology of overactive bladder is complex, resulting from both neurogenic and myogenic disturbances. According to myogenic OAB theory, bladder overactivity develops as a result of abnormal coupling and myoelectrical activity of the smooth muscles [3]. However, it is the theory of disturbed nervous system control that is strongly favoured with regards to the pathogenesis of AOB. Probably, a combination of disturbances in both the central and peripheral neural mechanisms is responsible for urgency and other OAB symptoms. These mechanisms involve both central and peripheral sensory (increased bladder receptor excitability and/or afferent bladder output, abnormal central processing of afferent discharge) as well as motor (increased smooth muscle contractility induced by neural and non-neural stimuli, involuntary immoderate parasympathetic nerve supplying reflexes) aspects [3,8]. Moreover, the current pathophysiology of OAB emphasizes the role of the urothelium, a multifunctional tissue that not only acts as a barrier between the bladder contents and the underlying tissues, but is also considered

to be a sensory organ, transducing physical and chemical stimuli to the attendant afferent nerves and smooth muscles of the bladder [5].

Since the pathogenesis of OAB has not been fully elucidated so far, treatment is aimed at relieving the symptoms rather than curing. There are several treatment options, including conservative treatment, oral pharmacological agents, intravesical therapy, or surgery [17]. The potential sites for pharmacological intervention consist of the smooth muscles of the bladder, efferent/afferent nerves, and the central nervous system. The current, typical pharmacological management of OAB involves antimuscarinics (anticholinergic medications) as a result of their confirmed inhibitory role with regards to the muscarinic receptors. Human bladder mostly contains M2 and M3 receptors; it is postulated that these receptors evoke contraction of the smooth muscles and are involved in sensory (afferent) activation. Six antimuscarinics are approved for OAB treatment: oxybutynin, tolterodine, trospium, propiverine, solifenacin, and darifenacin. All have received excellent rating (level 1 – data from randomized controlled studies, grade A – highly recommended). However, antimuscarinics can produce unwanted side effects (dry mouth, constipation, dizziness, and headache) [17,39]. Thus, OAB management continues to evolve with the development of a number of new concepts.

$\beta$ -3 adrenoceptor agonists are among the new concepts considered during the development of new AOB management options. It has been discovered that the stimulation of  $\beta$ -3 receptor results in an increase in the heart rate, lipolysis with higher energy expenditure, and improved glycemic control [33]. Thus,  $\beta$ -3 agonists have been considered as therapeutic agents for experimental obesity, type 2 diabetes, and heart failure treatment; however, the results of the clinical trials have not been encouraging [33]. Rat  $\beta$ -3 adrenoceptors were found to be expressed in brown and white adipocytes, liver, skeletal muscle, ileum, colon, and brain. The presence of  $\beta$ -3 adrenoceptors in humans has been confirmed in adipose, small intestine, colon, stomach, heart, and brain [16]. Moreover,  $\beta$ -3 adrenoceptors have been identified in both rat and human bladders and stimulation of  $\beta$ -3 adrenoceptor is thought to be a valuable approach for the treatment of frequent urination and urinary incontinence, because there is evidence indicating that detrusor relaxation is mediated through activation of this receptor [16,33]. The signalling pathway of  $\beta$ -3 adrenoceptors includes the activation of adenylyl cyclase with the subsequent cAMP formation. Nevertheless, it seems that this mechanism does not have a crucial contribution to adrenomimetic-mediated bladder relaxation. Bigger importance for  $\beta$ -3 agonists-mediated bladder relaxation is attributed to the activation of potassium channels, leading to the efflux of potassium, hyperpolarisation, and reduced tone of the smooth muscle cells [14,19]. Increased cAMP activates protein kinase A and finally results in large-conductance calcium activated potassium BK channels excitation. Sev-

eral types of potassium channels have been investigated in bladder tissues, including voltage-gated, small conductance, ATP-sensitive, and large conductance, with the latter demonstrated to possess the predominant role [14]. In general, calcium channels classified into dihydropyridine-sensitive L-type voltage-gated ones are responsible for depolarisation, intracellular calcium influx and following urinary bladder smooth muscle contractions. The repolarisation period is regulated mainly by BK potassium channels mentioned above. There are evidences that the activation of  $\beta$ -3 adrenoceptors by BRL37344 and other agonists involves increased Ca-dependent smooth muscles hyperpolarisation and their relaxation [1,19]. Moreover, apart from direct action on bladder smooth muscle, the relaxing effect of  $\beta$ -3 adrenoceptor agonists may also result from the release of urothelium-derived nitric oxide (NO). The indirect confirmation on the role of NO in bladder filling are the results confirming that intravesical administration of NO donors diminishes detrusor hyperactivity, whereas oxyhemoglobin (NO scavenger) stimulates bladder activity [14].

It is also worth remembering that relaxing action of NO, secondary to  $\beta$ -3 bladder adrenoceptors activation, may not be mediated by a direct action on smooth muscle cells, because they lack soluble guanylate cyclase – an important component in NO-mediated relaxation. Instead, NO may modulate bladder reflexes by altering activity of the afferents. This mechanism of  $\beta$ -3 adrenoceptors-evoked bladder relaxation provides further insights into the role of urothelial cells in the sensory functions of the bladder [4,5].

In conclusion, there are many premises supporting the concept that selective agonistic impact on  $\beta$ -3 adrenoceptors may be an effective pharmacological option to treat bladder over-contraction. The role of  $\beta$ -3 adrenoceptors and their agonists in management of bladder overactivity is currently under intense investigation. There are several reports demonstrating the beneficial role of  $\beta$ -3 agonists in animal OAB models (experimental conditions with the presence of bladder overactivity symptoms, which are thought to mirror human OAB; further explained in the text above). Woods et al. [41] found that CL316243, a selective  $\beta$ -3 adrenoceptors stimulant, inhibits the smooth muscle cells contractility in depolarized, isolated rat detrusor strips. Hicks et al. [18] confirmed the results of previously mentioned investigators, reporting that GW427353 (solabegron) also evokes bladder relaxation and facilitates bladder storage mechanisms in dog isolated bladder strips. Further experimental evidence was supplied by Kaidoh et al. [20] study, who demonstrated that the administration of CL316243 suppresses the detrusor hyperreflexia in infarcted rat brain. Another study by Kullmann et al. [24] revealed that in rat ovariectomy OAB model  $\beta$ -3 adrenoceptors agonist, BRL37344, decreases voiding frequency by 40 to 70%; thus, suggesting the potential role of  $\beta$ -3 adrenomimetic ligands in the treatment of bladder hyperactivity in this setting. The encouraging results

of experimental studies, not only with regards to OAB, introduced  $\beta$ -3 agonists to the clinical trials, enabling the assessment of their effectiveness and safety. At the present, several ligands have been investigated, including solabegron (GW427353; GlaxoSmithKline; completed phase II for OAB and irritable bowel treatment), amibegron (SR58611; Sanofi-Aventis; completed phase III for anxiety and depressive disorders treatment), mirabegron (YT178; Astellas), and ritobegron (KUC7483; Kissei), both studied for OAB treatment, as well as LY377604 (Elly Lilly) and L-796568 (Merck), which were tested as anti-obesity and type 2 diabetes compounds [33]. The preliminary results confirm the experimental findings, indicating that the studied  $\beta$ -3 agonists will be registered as new OAB medication in the close future. On the other hand, clinical trials also revealed a number of side effects of these agents; probably, limiting their wide usage in OAB treatment. For example, solabegron was found to produce visceral hyperalgesia by releasing somatostatin from adipocytes, while amibegron was demonstrated to produce abnormalities in hepatic functioning [33]. Moreover, BRL37344 was also noted to increase the levels of circulating transaminases, accompanied by dynamic changes in glucose metabolism [22].

Apart from the indisputable evidence confirming the effectiveness of  $\beta$ -3 agonists in OAB management, a number of pharmacological aspects of  $\beta$ -3 agonist's action need to be defined. For example, the effect of  $\beta$ -3 adrenoreceptor stimulation on the autonomic nervous system activity during OAB is still not fully explained. Therefore, the present study was designed to investigate the probable  $\beta$ -3 adrenoreceptor-mediated changes in autonomic nervous system (ANS) activity, following the administration of one of the  $\beta$ -3 agonists, BRL37344, in a rat OAB model evoked by long-lasting bladder outlet obstruction. ANS functioning can be assessed non-invasively using heart rate variability (HRV) method, based on the ECG signal. The fundamental assumption in HRV analysis is that the rhythmical sinus heart rate is influenced by both sympathetic and parasympathetic modulation, which is reflected by the main HRV parameter and referred to as the mean time between successive normal-normal intervals (mean NN), as well as numerous mean NN-derived secondary statistical parameters (time-domain HRV analysis). HRV is also analysed after transforming the primary ECG recording into the so called HRV spectrum (with its total power; TP), which can be divided according to the frequency range into some non-normalized (at very low frequencies – VLF, low – LF, and high – HF) components and normalized (nLF, nHF) parameters, reflecting global (TP), pure sympathetic (nLF), pure parasympathetic (nHF, HF), and both ANS branches (LF) tone [27,34].

## MATERIALS AND METHODS

**Ethics:** The study protocol was approved by the First Local Ethic Committee in Cracow (agreement decision 126/2010).

**Animals, studied groups and general study plan:** The animals (female 8-week-old Wistar rats) were obtained from the central laboratory. Upon arrival at the animal house at the Pathophysiology Department, the rats were allowed an acclimatisation period of one week in groups of five per cage. The animals were housed at room temperature, with 12-12 hours day-night cycle, with standard food (Labofeed Kcynia) and water ad libitum.

We performed our study using 30 rats. After the acclimatisation period, 20 animals (starting mean body weight  $181.67 \pm 7.82$  g) underwent surgically induced proximal bladder outlet obstruction (BOO; see below), while 10 sham-operated rats were enrolled into the control group. The BOO rats were then maintained for five weeks to allow for bladder overactivity due to developing bladder outlet obstruction (long-lasting bladder outlet obstruction; LLBOO). After this time, the animals were randomised into the non-treated group (LLBOO) and the  $\beta$ -3 agonist (BRL37344) treated group (LLBOO+BRL37344). The studied compound, BRL 37344, was obtained from Sigma Aldrich. We planned to perform HRV recordings with statistical control/LLBOO/LLBOO+BRL37344 differences analysis and bladder macroscopic and histological assessment in all studied animals. The final body weight at the end of the experiment was  $221.14 \pm 11.35$  g in LLBOO rats and  $259.51 \pm 8.22$  g in control animals.

**Long-lasting bladder outlet obstruction model:** 20 animals enrolled into the BOO group underwent surgery to induce partial urine outflow obstruction, leading to bladder overactivity. The technique used to obtain bladder outlet obstruction, both in growing and mature animals, has already been described in the 1980s by a number of investigators, for example, Sibley (1985) [36], and it is still used with small modifications by numerous researchers (e.g. Das et al.; 2002 [11], Kamiyama et al.; 2007 [21]). This experimental model is considered to produce similar bladder overactivity as that observed in OAB. Moreover, BOO animal model faithfully mimics bladder overactivity due to benign prostatic hyperplasia (BPH) in humans. It shows many of the structural and physiological bladder wall changes observed in human BOO, including muscle cells hypertrophy, altered responsiveness to various stimuli, increased myogenic activity, and enlarged sensory neurons with non-micturition contractions. Thus, partial long-lasting BOO appears to be a good model to recapitulate bladder overactivity symptoms; especially, those of bladder overactivity secondary to BPH [32].

The rats were briefly anaesthetised with sodium pentobarbital (Morbital, Biowet, Puławy; 35 mg/kg body weight) intraperitoneally (i.p.). Under general anaesthesia, a 1 mm diameter stainless urinary catheter was placed into the urethra. After performing a lower midline laparotomy, the bladder and proximal urethra were exposed and a 4/0 silk ligature was tied around the



proximal urethra and the inserted rod. Following this, the catheter was removed leaving the urethra partially occluded. The laparotomy incision was then closed, two antibiotics (neomycinum – Neomycin spray, oxytetracyclinum – Oxycort spray) were administrated through the surgery wound and the animals were allowed to recover. One of the operated animals died during the surgery and three others in the early postoperative period. Thus, in the end, we studied 16 LLBOO participants.

**LLBOO group:** 8 rats were randomised into the control group. Prior to HRV recordings, these animals were given i.p. 0.9% saline in the appropriate volume, similar to  $\beta$ -3 agonist treated animals.

**LLBOO+BRL37344 group ( $\beta$ -3 agonist treated group):** 8 rats were randomised into this group. The rats were administrated single i.p. dose of  $\beta$ -3 agonist, labelled in Sigma Aldrich as BRL37344 sodium salt hydrate (( $\pm$ )-(R\*,R\*)-[4-[2-[[2-(3-Chlorophenyl)-2-hydroxyethyl]amino]propyl]phenoxy]acetic acid sodium hydrate).

According to previously published Kullman et al. [24] study, we used BRL37344 dose of 5 mg/kg body weight, taking into consideration their findings confirming that this dose had a consistent and significant effect on voiding frequency. BRL37344 was dissolved in water and administrated prior to HRV recording.

**Control group:** 10 animals (starting body weight 180.34  $\pm$  6.82 g) were sham-operated without proximal urethra ligation. All animals from this group survived the operating intervention and were kept for the same time and in the same conditions as those from LLBOO group. HRV recordings in control rats were taken without the vehicle or  $\beta$ -3 agonist.

**HRV studies:** On the fifth week after BOO induction, HRV recording was taken in all studied animals. Firstly, animals were treated with vehicle (LLBOO group) or  $\beta$ -3 agonist (LLBOO+BRL37344). Control rats did not receive these agents. In LLBOO, HRV recordings were performed 2-4 hours after drug or vehicle treatment in compliance to Kullman et al. [24] findings, demonstrating that the effect of BRL37344 lasted for 4 to 6 hours.

In each studied animal, ECG recordings were taken under urethane anaesthesia (1200 mg/kg body weight; Sigma-Aldrich) during the 20-minute rest periods. This anaesthetic agent was chosen after taking into consideration literature reports suggesting proportional (up to the applied dose) impact on tonic activity of both the sympathetic and parasympathetic ANS elements and a relatively small influence on cardiac reflexes [25,26]. HRV analysis was performed after terminating ECG registration and eliminating extrasinusal extopics. Standard time (mean NN, max NN, min NN, SDNN – all in [ms]; rMSSD, mean HR [bpm]), spectral (frequency; TP, VLF, LF, HF – all in [ms\*ms]); and normalised

nLF and nHF in [n.u.]) parameters were calculated. The spectrum bands for respective components were set as: 0.18<VLF<0.28<LF<0.78<HF<3. Commonly accepted interpretation criteria were employed, according to HRV guidelines [2,27,34]. Results were presented as mean values  $\pm$  SD.

We accepted such frequency bands for the individual HRV components as it has been mentioned above according to previously published Cerrutti et al [7] and Aubert et al. [2] studies. However, depending on the studied experimental model and species of the studied animals, various frequency ranges determining HRV spectral components powers are accepted. Some of them are given in Rowan III et al review [34]. Thus, one should always remember about these methodological details when compared one's results to those ones obtained in another studies.

**Statistical HRV parameters analysis:** The statistical assessment of the results was performed in paired studied groups (control vs. LLBOO; control vs. LLBOO+BRL37344, and LLBOO vs. LLBOO+BRL37344) using parametric Student's t-test with  $\alpha$ =0.05. The H0 hypothesis of equality of analysed parameter variations in two studied populations was verified versus an alternative H1 hypothesis, which assumed their inequality (and thus the existence of statistically significant differences).

**Urinary bladder assessment:** In all studied rats, the bladders were collected once the HRV recordings had been taken and a lethal sodium pentobarbital dose (100 mg/kg body weight) was administered, in order to compare the changes in bladder wet weight (BWW) and its percentage related to final body weight in control and LLBOO animals. Bladders were cut off with the proximal ligated (LLBOO rats) or normal (controls) urethra. They were gently drained of their content using gauze and immediately weighted. According to literature, cystitis may be evaluated not only by determining the changes in macroscopic and microscopic analysis, but also via bladder wet weight. Thus, BWW is regarded as an indirect marker of cystitis and bladder dysfunction [31,35,42].

## RESULTS

**Bladder wet weight in studied groups:** BWW values did not differ in both control and obstructed animals. Mean bladder wet weight was 0.137  $\pm$  0.064 g (range 0.04 to 0.22 g) in sham operated controls vs. 0.147  $\pm$  0.058 g (range 0.11 to 0.34 g) in LLBOO rats.

Nonetheless, we revealed that although there was no difference in BWW in both control and LLBOO rats, the percentage of bladder wet weight in relation to final body weight differed in these populations (0.046  $\pm$  0.021 g vs. 0.066  $\pm$  0.024 g, respectively; p=0.04). Thus, BWW increase in LLBOO animals suggests rebuilding of the bladder wall and its dysfunction in this group.

The description of the LLBOO and control rats is given in table 1.

Table 1. The description of the LLBOO and control rats

	Control rats	Long-lasting bladder obstruction rats	Statistic
starting body weight [g]	180.34 ± 6.82	181.67 ± 7.82	NS
Final body weight [g]	259.51 ± 8.22	221.14 ± 11.35	NS
bladder wet weight [g]	0.137 ± 0.064	0.147 ± 0.058	NS
bladder wet weight percentage related to final body weight [%]	0.046 ± 0.021	0.066 ± 0.024	p = 0.04

### HRV time-domain parameters

**The comparison between our control and literature-based control rats:** The values of the time-domain parameters obtained in our study are partly similar to those ones found by Aubert et al [2]. We revealed mean NN as 163.41 [ms] and min NN as 139.28 [ms] and these results were in concordance with their study (mean NN – 174.20 [ms] and min NN – 131.0 [ms]). On the other hand, however, we found a lower max NN result (188.76 [ms] vs. 213.0 [ms]) and higher rMSSD value (13.01 [ms] vs. 5.2 [ms]) comparing to Aubert's study [2].

**The comparison between control and both treated and non-treated LLBOO rats:** Mean NN did not differ in comparison between the control and LLBOO rats, both treated and non-treated. Analysing control and non-treated LLBOO populations, we revealed statistically higher range, SDNN, and rMSSD values in control rats (49.47 ± 6.47 vs. 31.06 ± 9.41; 8.70±2.73 vs. 4.13 ± 1.68;

13.01±7.35 vs. 1.33 ± 0.39; respectively). We found no essential differences comparing time-domain HRV parameters in control and BRL-treated rats, except rMSSD value, which was significantly higher in the control group (13.01 ± 7.35 vs. 6.02 ± 3.29).

**The comparison between LLBOO and LLBOO treated rats:** Mean NN interval and its maximal value, together with average heart rate did not differ significantly in both groups. Minimal NN reached higher values in control rats (153.58 ± 11.72) as compared to β-3 treated animals (140.07 ± 1.24; p=0.03). The latter group also displayed higher values of both SDNN (7.30 ± 1.55 vs. 4.13 ± 1.68 in the controls; p=0.01) and rMSSD (6.02 ± 3.29 vs. 1.33 ± 0.39 in controls; p=0.02).

When analysing the general trend observed in time-domain HRV parameters in BRL37344 treated group, it should be emphasised that some of those mentioned above (mean NN, max NN, average HR) were almost identical when compared to the controls, while others were increased. The summary of time-domain HRV parameters is given in table 2.

### HRV spectral-domain parameters:

**The comparison between our control and literature-based control rats:** The spectral components values demonstrated in our study were lower comparing to those ones revealed by Aubert et al [2] (our results: TP-36.09 [ms<sup>2</sup>]; LF-5.40 [ms<sup>2</sup>]; HF-11.47 [ms<sup>2</sup>] vs. 78.98; 18.42 and 15.66; respectively). The differences may be due to both various time of HRV recordings (we collected ECG signal for 20 minutes while Aubert et al [2] did it for 30-minutes) and the studied animals status (we performed HRV recordings in rats under urethane anaesthesia while they studied unrestrained animals). These methodological aspects might contribute to HRV spectrum power augmentation in Aubert et al. [2] study.

**The comparison between control and both treated and non-treated LLBOO rats:** Most values of HRV spectral parameters were considerably dif-

Table 2. Time-domain HRV analysis results

	1 - Control group	2 - LLBOO group	3 - LLBOO+BRL37344	Statistic p value		
				1-2	1-3	2-3
mean NN [ms]	163.41±10.27	171.94 ± 14.84	170.77 ± 6.26	NS	NS	NS
max NN [ms]	188.76±6.28	184.62 ± 9.53	187.02 ± 2.12	NS	NS	NS
min NN [ms]	139.28±6.36	153.58 ± 11.72	140.07 ± 1.24	0.02	NS	0.03
range [ms]	49.47±6.47	31.06 ± 9.41	46.94 ± 2.16	0.006	NS	0.01
average HR [bpm]	368.32±22.55	351.32 ± 34.14	351.42 ± 12.97	NS	NS	NS
SDNN	8.70±2.73	4.13 ± 1.68	7.30 ± 1.55	0.008	NS	0.01
rMSSD	13.01±7.35	1.33 ± 0.39	6.02 ± 3.29	0.01	0.05	0.02

ferent in control and LLBOO, non-treated animals. We revealed a deep fall in all non-normalized spectral parameters, together with HRV total power in LLBOO rats. Moreover, the normalized spectral components were also different in these two populations, with nLF/nHF quotient of about 1/2 in the control group ( $36.61 \pm 16.04/63.39 \pm 16.04$ ; respectively) and 1/4 in LLBOO rats ( $19.67 \pm 9.59/80.33 \pm 9.59$ ). When the controls were compared to LLBOO+BRL37344 groups, a similar tendency of statistically significant, diminished non-normalized spectral parameters was observed (except VLF), although somewhat higher values were reached in LLBOO-β-3 agonist treated animals then in the non-treated rats. In the contrast to the findings mentioned above, no differences related to normalized spectral parameters were displayed in the control - LLBOO+BRL37344 populations.

**The comparison between LLBOO and LLBOO treated rats:** The statistical estimation of calculated spectral HRV analysis parameters indicated differences between β-3 agonist treated and non-treated animals,

relating to almost all calculated parameters, both non-normalized (TP, VLF) and normalized (nLF, nHF). Both TP ( $12.21 \pm 11.88$ ) and VLF ( $9.64 \pm 9.80$ ) were considerably higher in BRL37344 treated animals, as compared to the controls ( $2.68 \pm 1.29$  and  $2.06 \pm 1.02$ ; respectively;  $p=0.05$ ). Moreover, a considerable increase in LF/HF ratio was revealed in this group ( $0.63 \pm 0.44$  vs.  $0.26 \pm 0.16$ ). We also noted an increase in LF and HF powers in β-3 treated animals, as compared to the controls, but it was statistically insignificant. Conversely, normalised nLF value was significantly higher ( $34.77 \pm 17.52$ ) and nHF lower ( $65.23 \pm 17.52$ ) in β-3 agonist treated group when compared with the control ( $19.67 \pm 9.59$  and  $80.33 \pm 9.59$ , respectively;  $p=0.05$ ).

The HRV spectral analysis detailed results mentioned above are given in table 2 and have been also presented as a visual summary in the figures 1 and 2.

Moreover, we calculated the percentage of individual power spectral components in total HRV power in both

Table 3. Spectral-domain HRV analysis results

	1 - Control group	2 - LLBOO group	3 - LLBOO+BRL37344	Statistic		
				1-2	1-3	2-3
TP [ms <sup>2</sup> ]	36.09±17.83	2.68 ± 1.29	12.21 ± 11.88	0.007	0.02	0.05
VLF [ms <sup>2</sup> ]	19.23±15.74	2.06 ± 1.02	9.64 ± 9.80	0.04	NS	0.05
LF [ms <sup>2</sup> ]	5.40±2.54	0.11 ± 0.06	1.03 ± 1.22	0.005	0.007	NS
HF [ms <sup>2</sup> ]	11.47±8.88	0.51 ± 0.38	1.55 ± 1.28	0.03	0.03	NS
LF/HF	0.70±0.63	0.26 ± 0.16	0.63 ± 0.44	NS	NS	0.05
nLF [n.u.]	36.61±16.04	19.67 ± 9.59	34.77 ± 17.52	0.04	NS	0.05
nHF [n.u.]	63.39±16.04	80.33 ± 9.59	65.23 ± 17.52	0.04	NS	0.05

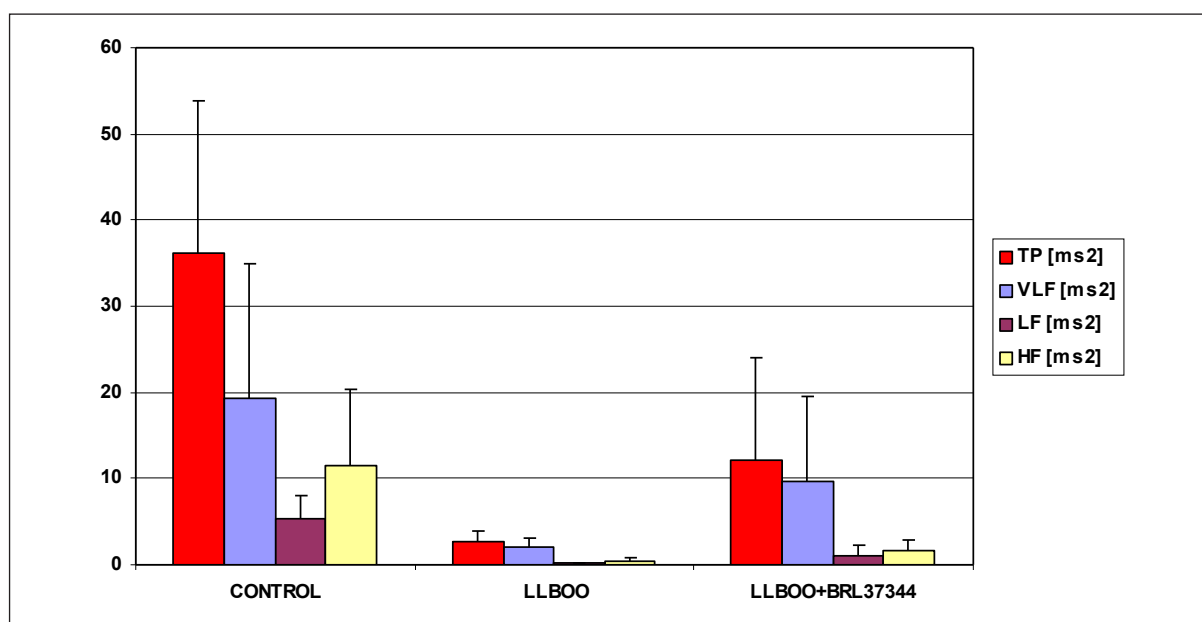


Fig. 1. HRV spectrum comparison in studied groups (detailed results are given in table 3)

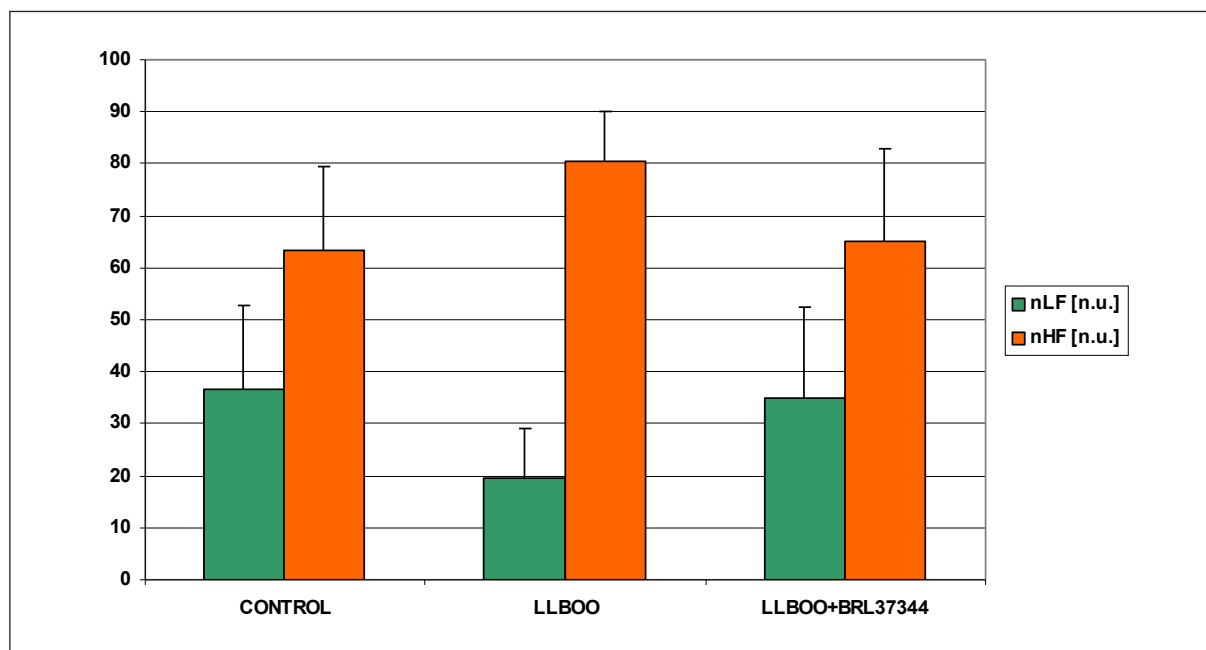


Fig. 2. HRV normalised spectral components in the studied groups (detailed results are given in table 3)

analysed groups. In control animals, the disproportion between three non-normalized spectral components was smaller than in other groups. We found that the percentage of LF differed considerably in both LLBOO populations. In LLBOO non-treated animals, this component amounted to about 4.21%, whereas in BRL37344 treated animals this parameter reached an almost twice higher value, 8.51%, in relation to the appropriate total powers. Contrary to LF% differences, the remaining non-normalized spectral components (VLF, HF) achieved nearly the same percentages related to the appropriate total powers (%VLF – 76.62 in LLBOO animals and 70.41 in the tested drug treated group, while %HF was 19.81 and 21.09, respectively).

The distributions of the individual HRV non-normalized spectral components in all studied groups are given in figures 3-5.

## DISCUSSION

The main finding of our study was to demonstrate the autonomic nervous system disturbances in long-lasting experimental bladder outlet obstruction (LLBOO) model. We revealed that:

1. LLBOO animals were characterized by diminished NN range, SDNN, and rMSSD in time-domain analysis as compared to the control. Similarly, total power and non-normalized spectral HRV parameters were also decreased. Contrary to these findings, normalized spectral parameters were lower (nLF) and higher (nHF) than in the controls. These findings suggest a global diminishing in ANS activity, with marked parasympathetic predominance, which may be at least co-responsible for bladder overactivity during the filling period.

2. The animals treated with  $\beta$ -3 agonist, BRL37344, displayed similar HRV results as those observed in the controls. We demonstrated no significant differences in time-domain HRV parameters compared to the control, except that rMSSD was still much lower in treated rats. In spectral analysis, a power decrease in LF and HF, together with a fall in TP, were found when compared to the control; although, TP and VLF achieved higher values in BRL37344-treated animals than in those that did not receive treatment. Notably, both normalized spectral parameters (nLF and nHF) reached almost the same values in control and  $\beta$ -3 agonist-treated rats. All of these findings suggest that following the administration of  $\beta$ -3 agonist, the global activity and functioning of ANS was improved; in particular, there was a markedly smaller disproportion between the sympathetic and parasympathetic tone in this group.

3. Our results, which are in agreement with literature reports [18,20,24,41], suggest that  $\beta$ -3 agonist (BRL37344) seems not only to ameliorate bladder overactivity during the storage period, but may be also considered as an agent abolishing the autonomic imbalance present in the course of LLBOO. Thus, the restoration of appropriate autonomic activity may contribute to relieving the symptoms of bladder overactivity in  $\beta$ -3 treated patients.

The results of sparse clinical reports estimating the functional condition of ANS appear to be ambiguous and conflicting. These discrepancies are due to differences in methodological approach of the investigations (some of them are based on short-term ECG recordings; whereas, others on 24-hourlong Holter ECG registrations). Moreover, the studies were con-



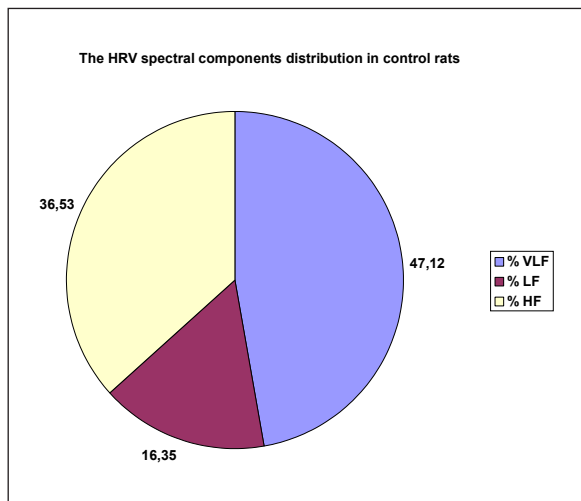


Fig. 3. The distribution of non-normalized HRV spectral components in control group

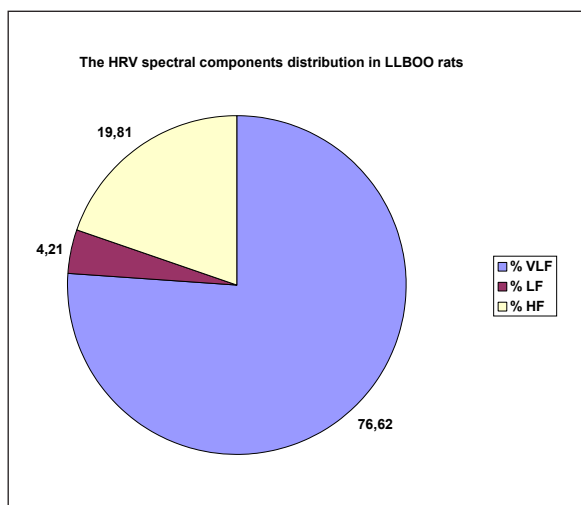


Fig. 4. The distribution of non-normalized HRV spectral components in LLBOO group

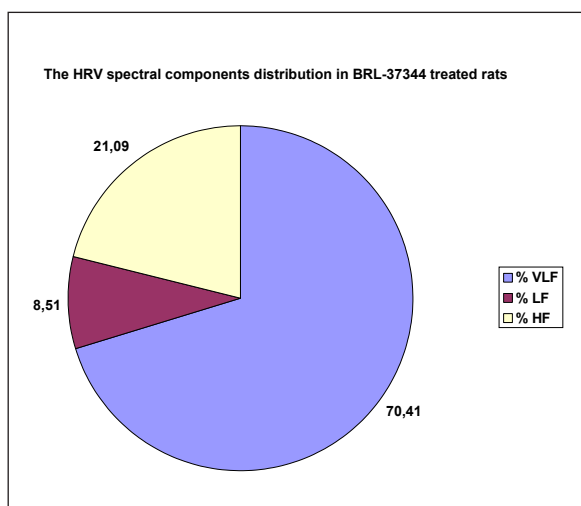


Fig. 5. The distribution of non-normalized HRV spectral components in LLBOO+BRL37344 group

ducted on two populations of BPH patients, which differed with regards to their pathophysiological conditions (BPH patients with BPH accompanying metabolic syndrome or isolated BPH patients). The high sympathetic activity demonstrated in the metabolic syndrome may also constitute the cause of prostate growth. There are reports confirming such dependence [9,28]. In one of our previous clinical studies, we also obtained results confirming sympathetic overactivity in resting conditions in BPH patients [37]. According to these findings, the patients should be treated using  $\alpha$ -1 adrenergic agents, reducing the degree of prostate hypertrophy and urethra compression by the prostatic smooth muscles [10,29]. This is especially important considering that  $\alpha$ -adrenergic receptors were found to be present in the urinary bladder. The  $\alpha$ -adrenergic receptors, in contrast to  $\beta$ -adrenergic receptors, contribute to bladder contraction; although, to a markedly smaller degree than M1 and M3 muscarinic receptors. Consequently, the abovementioned ideas substantiate  $\alpha$ -adrenergic blockade as a potential therapeutic option. Moreover, antagonistic influence on  $\alpha$ -adrenoreceptors improves bladder blood flow and protects against ischaemic changes [10]. Nevertheless, there is a subpopulation of BPH patients with findings of different autonomic disturbances. Kim et al. [23] showed a decrease in TP and HF in short-term HRV recordings, without changes in the remaining non-normalized spectral components. Conversely, Zorba et al. [43], in one of the recently published works, revealed an obvious increase in all non-normalized HRV components (VLF, LF, and HF), although this increase was not statistically significant.

It is difficult to compare the results of experimental and clinical reports, not only with regards to the methodological differences, but also taking into account physiological conditions (different rat and human resting heart rate, different spectral bands for basic HRV components). Therefore, we have reservations about the above-cited papers; although, our results are in agreement with the conclusions formulated by Kim et al. [23].

Using a similar experimental model of bladder outlet obstruction (although shorter, lasting only 2 weeks), we have also showed a total decrease in HRV power, together with a decline in the two standardised components – LF and HF, in our previous study, published this year [13]. The value of rMSSD parameter was also lower in the studied group as compared to the control animals. The distribution of the individual HRV power components demonstrated the growth of VLF percentage (to 90%) in BOO participants, in comparison to the control group, which showed a lower VLF percentage (only 53%) [13].

Our present study has corroborated the results of our abovementioned studies and confirmed the obvious intensification of autonomic disturbances to be present

also in the experimental model of long-lasting bladder outlet obstruction. We postulate that the present results prove deep impairment of ANS in LLBOO model. According to literature, the sympathetic tone is expressed through HR, SDNN, and LF, while parasympathetic tone is reflected by HF [27,34]. Taking into consideration these guidelines, our results confirm a decrease in the activity of both autonomic branches in LLBOO group. Moreover, we strongly emphasize that the results of normalized spectral components (nLF, nHF) in LLBOO group seem to be of particular significance, because they suggest considerable autonomic imbalance accompanied by parasympathetic predominance. It is hard to predict if autonomic changes revealed in our study are one of the possible causative OAB factors, intensifying bladder overactivity, or are a “compensatory” adaptive reaction of the bladder, enabling an improvement in urine outflow by higher contractility.

As a side note, it should be noted that we also showed a considerable decrease in VLF in LLBOO. At present, it is hard to interpret this finding, because both the physiological and pathophysiological mechanisms responsible for the origination of VLF component remain unclear. Despite the fact that VLF constitutes the decidedly largest part of the total HRV power, the significance of this component remains ambiguous. VLF is thought to reflect thermoregulatory mechanisms, fluctuations in renin-angiotensin-aldosterone system activity, or the function of peripheral chemoreceptors. Thus, VLF may exert not only cardiac stress, but general systemic stress as well [23,27]. In our experiment, this component achieved slightly lower percentage value in LLBOO animals (about 76%) as compared to previously mentioned short-term BOO subjects (90%); although, still it was the largest part of the total HRV power (both in LLBOO and control rats).

As mentioned in the introduction,  $\beta$ -3 agonists constitute the presently studied group of agents with the potential use in the treatment of OAB; their effectiveness

in addressing this clinical entity has been confirmed by reports [18,20,24,41]. We showed that the administration of  $\beta$ -3 agonist (BRL37344) is associated with an improvement in autonomic disturbances (understood as an increase in the total HRV power and its individual components). Although TP, LF, and HF have consistently remained lower in LLBOO rats as compared to the control group, there was a marked and perceptible trend in their growth. The key finding of our study was revealing the lack of the essential differences in normalized parameters (nLF and nHF) in animals treated with BRL37344 in comparison to the corresponding results in the controls. The activation of  $\beta$ -3 adrenoceptors was mirrored by the growth in nLF (from  $19.67 \pm 9.59$  in LLBOO non-treated group to  $34.77 \pm 17.52$  in  $\beta$ -3 treated group) and the fall in nHF (from  $80.33 \pm 9.59$  to  $65.23 \pm 17.52$ , respectively). Therefore, the administration of  $\beta$ -3 agonist decreased the considerable disproportion between sympathetic and parasympathetic tone, resulting in the restoration of sympathetic increase in the total HRV power. It may reflect the diminished cholinergic-mediated contractility of the bladder and the augmented adrenergic-evoked bladder relaxation.

Moreover, there are evidences demonstrating that long-term bladder outlet obstruction causes diminished density of the bladder  $\beta$ -3 adrenoceptors and, therefore, results in decrease of the inhibitory bladder response mediated by these receptors [19]. Thus, the increase of the sympathetic tension after BRL37344 may contribute to the relatively enlarged stimulation of the still remaining  $\beta$ -3 adrenoceptors and bladder smooth muscles relaxation.

In conclusion, our study revealed that the beneficial effect of the studied  $\beta$ -3 agonists may also result from correcting autonomic disturbances. In order to establish the mechanism of OAB amelioration using  $\beta$ -3 agonists, further research, requiring a larger number of studied animals and other  $\beta$ -3 adrenergic agents, is needed.

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