Life Sciences 84 (2009) 719–724

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Heart rate and arterial pressure variability and baroreflex sensitivity in ovariectomized spontaneously hypertensive rats

Valdo José Dias da Silva [a,*], Rodrigo Miranda [a], Luciano Oliveira [a], Carlos Henrique F. Rodrigues Alves [a], Glaucia Helana Fortes Van Gils [b], Alberto Porta [c], Nicola Montano [c]

[a] School of Medicine, Federal University of the Triangulo Mineiro, Uberaba, MG, Brazil
[b] Department of Physiology, University of Uberaba, Uberaba, MG, Brazil
[c] Department of Clinical Sciences, Internal Medicine II, Luigi Sacco Hospital, University of Milan, Milan, Italy

ARTICLE INFO

Article history:
Received 4 June 2008
Accepted 16 February 2009

Keywords:
Estrogen
SHR
Heart rate variability
Arterial pressure variability
Power spectral analysis
Baroreflex

ABSTRACT

Aims: The present study evaluated the effects of ovariectomy on heart rate and arterial pressure variability and cardiac baroreflex sensitivity (BRS) in female spontaneously hypertensive (SHR) and Wistar–Kyoto rats (WKY).

Main methods: Sham-surgery animals were used as control. Sixteen weeks after ovariectomy or sham-surgery, animals were recorded. Time series of pulse interval (PI) and systolic AP (SAP) were analyzed by means of autoregressive spectral analysis, which quantifies the power of very low (VLF = 0.01–0.25 Hz), low (LF = 0.25–0.75 Hz) and high frequency (HF = 0.75–2.5 Hz) bands. BRS was assessed by means of linear regression between changes of PI and SAP induced by vasoactive drugs or calculation of α-index, a spontaneous BRS index.

Key findings: There was no difference in baseline PI or SAP between ovariectomized and sham SHR. Spectral analysis of heart rate variability suggested a shift of sympatho-vagal balance toward sympathetic predominance in ovariectomized SHR (LF/HF = 1.8 ± 0.2 versus 0.7 ± 0.2 in sham SHR, $p<0.05$). Ovariectomy increased total variance and VLF power of SAP in SHR (29.1 ± 9.6 $mmHg^2$ and 18.6 ± 6.3 $mmHg^2$ versus 9.1 ± 2.1 $mmHg^2$ and 4.3 ± 1.4 $mmHg^2$, respectively, in sham SHR, $p<0.05$). In addition, ovariectomy reduced reflex bradycardia in SHR (0.18 ± 0.03 ms/mmHg versus 0.34 ± 0.06 ms/mmHg in sham SHR, $p<0.05$). Ovariectomy did not affect heart rate and SAP variability or BRS in WKY.

Significance: These data showed that ovarian hormones deprivation induced marked changes on cardiovascular control, increasing SAP variability and cardiac sympatho-vagal balance and blunting BRS in female hypertensive animals, which reinforce the possible protective role of ovarian hormones on the cardiovascular system.

Introduction

Epidemiological and clinical studies have indicated that the incidence of cardiovascular diseases is less pronounced in young pre-menopausal women as compared to same age men (Becker and Corrao 1990; Hayward et al. 2000). This difference decreases with aging and disappears after menopause, when cardiovascular disorders become an important cause of death among women (Becker and Corrao 1990; Hayward et al. 2000). These data strongly suggest that ovarian hormones play a protective role of the cardiovascular system of young women (Becker and Corrao 1990; Dahlberg 1990; Hayward et al. 2000). However, the mechanism(s) of this protective effect is (are) not completely understood and is (are) the subject of intensive studies.

Remarkable metabolic effects on plasma lipoprotein levels, as well as a protective role on endothelial function, have been considered to be the most effective mechanisms of the cardio-protective action of estrogen (Becker and Corrao 1990; Hayward et al. 2000). In addition, clinical and experimental studies have suggested the existence of gender-related differences in the autonomic control of cardiovascular modulation (Sato et al. 1995; Huikuri et al. 1996; Saeki et al. 1997; Dart et al. 2002; Leicht et al. 2003). Estrogen seems to be able to increase vagal parasympathetic activity to the heart as well as to decrease sympathetic drive to different vascular beds (Huikuri et al. 1996; Saleh and Connell 1999; Saleh et al. 2000; Dart et al. 2002).

Studies of heart rate variability (HRV) and cardiac baroreflex sensitivity (BRS) have been widely used as an indirect assessment of cardiac sympatho-vagal balance and cardiac parasympathetic reflex control, respectively (Malliani et al. 1991; Montano et al. 1994; Task Force 1996; La Rovere et al. 2001). These approaches have been used

* Corresponding author. Department of Biological Sciences, Federal University of the Triangulo Mineiro, Praça Manoel Terra, 330, Uberaba, MG, 38015-050, Brazil. Tel.: +55 34 3318 5421; fax: +55 34 3318 5466.
E-mail address: valdo@mednet.com.br (V.J. Dias da Silva).

0024-3205/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2009.02.019

to stratify cardiovascular risk of life-threatening cardiac arrhythmias and sudden death (Malliani et al. 1991; Task Force 1996; La Rovere et al. 2001). HRV and BRS are attenuated in postmenopausal women as compared to young women (Lipsitz et al. 1995; Huikuri et al. 1996). On the other hand, hormone replacement therapy with estrogen is able to improve the indices of HRV and BRS in post-menopausal women (Lipsitz et al. 1995; Huikuri et al. 1996; Dart et al. 2002).

Although the causes of increased postmenopausal morbidity and mortality are unclear, this increase may also involve changes in arterial pressure and its regulation associated with estrogen loss. For instance, the risk for arterial hypertension rises markedly after menopause (Dubey et al. 2002). Furthermore, taking into consideration that arterial hypertension is an ubiquitous cardiovascular disorder, and that it is very common to find hypertensive women during the menopause period (Dubey et al. 2002), it may be hypothesized that reduction of estrogen levels after menopause could be more devastating to the cardiovascular system. However, little is known about the effects of low estrogen levels on arterial hypertension and its autonomic regulation.

Therefore, the aim of the present investigation was to test the hypothesis that surgical removal of the ovarian glands (ovariectomy) has marked effects on basal heart rate and arterial pressure variability in the time and frequency domain, as well as cardiac baroreflex sensitivity in female spontaneously hypertensive rats (SHR) as compared to normotensive Wistar–Kyoto (WKY) rats.

Materials and methods

Twelve-week-old female SHR and WKY rats were provided by the Department of Biological Sciences of the School of Medicine of Triângulo Mineiro. The animals were housed under controlled temperature and lighting (12/12 h light/dark cycle), with free access to chow and tap water. All experimental procedures were in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996).

SHR and WKY rats were initially submitted to ovariectomy (OVX) or sham surgery (SHAM) and, after surgical recovery, were divided into four experimental groups: SHR-OVX ($n=7$), SHR-SHAM ($n=7$), WKY-OVX ($n=8$) and WKY-SHAM ($n=9$). Ovariectomy was performed under anesthesia (sodium pentobarbital, 40 mg/kg, i.p.). Briefly, after a bilateral flank incision, the ovarian glands were exposed and sectioned at the fallopian tube level. The uterus was returned into the abdominal cavity and the muscles and skin were sutured closed. The same procedure, except the removal of ovarian glands, was performed during sham surgery. The rats were then housed in individual cages during sixteen weeks. In the last four weeks, a vaginal smear was collected daily to verify the regularity of the estrous cycle of sham rats and the absence of the estrous cycle (permanent diestrous) in ovariectomized rats. Permanent diestrous in ovariectomized rats was used as an index of the efficacy of ovariectomy.

At the end of the sixteen week period, the animals were implanted, under anesthesia (sodium pentobarbital, 40 mg/kg, i.p.), with polyethylene catheters into the femoral artery and vein for direct measurement of arterial pressure and drug administration. The catheters were tunneled under the skin and were exteriorized on the back of the neck. In sham ovariectomized rats, this surgical procedure was performed always 24 h before the estrus phase of the estrous cycle. After the surgical procedures the animals were allowed to recover in individual cages for at least 24 h.

One day after the vessel catheterization, without the effect of anesthesia, the experiments were carried out on freely moving rats. The arterial catheter was connected to a pressure transducer (Statham P23Gb, Hato Rey, PR) and the amplified (Hewlett-Packard amplifier, model 8805A, Waltham, MS) signal was continuously sampled (1000 Hz) in an IBM/PC equipped with a 12 bit analog to digital board (CAD12/36 Lynx Eletrônica, São Paulo, Brazil). After recording the basal pulsatile arterial pressure (AP) during a 30 minute period the animals received, randomly, bolus injections (i.v.) of phenylephrine (0.25–8.0 μg/kg) or sodium nitroprusside (1.0–32.0 μg/kg), in a volume of 100 μL/kg for each dose, to elicit changes in AP to measure BRS, which was evaluated by the slope of the regression line obtained by best-fit points relating changes in pulse interval (PI) and systolic arterial pressure (SAP) (Head and McCarty 1987).

Pulsatile AP was processed with a customized software that determines beat-by-beat values of systolic (SAP) and diastolic arterial pressures and PI. The SAP and PI variability in the frequency domain was assessed by autoregressive spectral analysis as described elsewhere (Malliani et al. 1991; Rubini et al. 1993; Task Force 1996). Briefly, a modeling of the oscillatory components present in the time series of SAP and PI was calculated based on the Levinson–Durbin recursion, with the order of the model chosen according to Akaike's criterion (Malliani et al. 1991; Rubini et al. 1993; Task Force 1996). This procedure allows an automatic quantification of the center frequency and power of each relevant oscillatory component present in the time series. The oscillatory components were labeled as very low (VLF), low (LF) or high frequency (HF) when their central frequency was located in a band of 0.01–0.25 Hz, 0.25–0.75 Hz or 0.75–2.50 Hz, respectively (Rubini et al. 1993). The power of the LF and HF components of HRV was also expressed in normalized units, obtained by calculating the percentage of the LF and HF variability with respect to the total power (all components from zero to 2.5 Hz) after subtracting the power of the very-low-frequency component (frequencies <0.25 Hz). The normalization procedure tends to minimize the effect of the changes in total power on the absolute values of LF and HF components of HRV (Malliani et al. 1991; Rubini et al. 1993; Task Force 1996).

Spontaneous BRS was calculated using the α-index within the LF range (= square root of the LF_{PI}/LF_{SAP} ratio). The calculation of the α-index requires the evaluation of coherence and phase between the PI and SAP time series. The bivariate autoregressive identification procedure (Pagani et al. 1988) was used to calculate the coherence (k^2) and phase shift (φ) between the PI and SAP time series. The coherence function measures the degree of linear correlation between the oscillations at the same frequency in both variability signals, while the phase shift measures the time lag or lead between the signals. In the LF frequency range, the coherence between PI and SAP is an expression of the baroreflex control of the heart rate (Pagani et al. 1988). The α-index was calculated in all cases since the coherence value was always significant ($k^2>0.5$) and the phase shift in radians was negative ($\varphi<0$ radians, i.e., SAP changes precede PI changes) (Pagani et al. 1988).

Table 1
Baseline values of body weight, pulse interval (PI), systolic (SAP), diastolic (DAP) and mean arterial pressure (MAP) in WKY and SHR submitted to sham (SHAM) or ovariectomy surgery (OVX).

	WKY		SHR	
	SHAM	OVX	SHAM	OVX
	($n=8$)	($n=9$)	($n=7$)	($n=7$)
Body weight (g)	369 ± 10	375 ± 11	295 ± 9*	315 ± 7*
PI (ms)	174 ± 5	185 ± 8	153 ± 4*	153 ± 4*
SAP (mmHg)	130 ± 2	124 ± 2	183 ± 5*	193 ± 7*
DAP (mmHg)	82 ± 2	95 ± 3	144 ± 6*	141 ± 5*
MAP (mmHg)	98 ± 2	104 ± 2	157 ± 4*	159 ± 4*

All values are expressed as mean ± SEM.
*$p<0.05$ versus WKY-SHAM.

Table 2
Mean values of spectral parameters and spontaneous baroreflex sensitivity (BRS) calculated for the systolic arterial pressure (SAP) and pulse interval (PI) time series using autoregressive spectral analysis.

	WKY		SHR	
	SHAM	OVX	SHAM	OVX
	($n=8$)	($n=9$)	($n=7$)	($n=7$)
PI				
Variance (ms^2)	12.5 ± 2.7	17.2 ± 6.5	10.8 ± 3.5	16.7 ± 4.2
VLF (ms^2)	7.9 ± 2.6	9.5 ± 4.5	7.6 ± 2.9	12.3 ± 3.8
LF (ms^2)	0.9 ± 0.2	1.3 ± 0.6	1.1 ± 0.3	2.2 ± 0.6
LF (nu)	38.1 ± 3.5	35.3 ± 7.3	36.9 ± 5.7	61.9 ± 3.5$^+$
HF (ms^2)	1.4 ± 0.2	2.6 ± 1.0	1.7 ± 0.4	1.2 ± 0.2
HF (nu)	61.5 ± 3.5	64.7 ± 7.3	63.1 ± 5.7	38.0 ± 3.5$^+$
LF/HF ratio	0.7 ± 0.1	0.7 ± 0.2	0.7 ± 0.2	1.8 ± 0.2$^+$
SAP				
Variance ($mmHg^2$)	7.2 ± 0.7	4.5 ± 1.3	9.1 ± 2.1	29.1 ± 9.6$^+$
VLF (ms^2)	2.6 ± 0.6	1.4 ± 0.6	4.3 ± 1.4	18.6 ± 6.3$^+$
LF ($mmHg^2$)	2.5 ± 0.4	1.7 ± 0.6	3.0 ± 1.1	7.9 ± 3.5
HF ($mmHg^2$)	1.4 ± 0.5	1.0 ± 0.3	1.1 ± 0.2	2.7 ± 0.9
Spontaneous BRS				
α-index	0.75 ± 0.04	0.81 ± 0.05	0.58 ± 0.03*	0.41 ± 0.02$^+$

All values are expressed as mean ± SEM.
*$p<0.05$ versus WKY-SHAM; $^+p<0.05$ versus SHR-SHAM. nu = normalized units.

Data are expressed as mean ± SEM. To evaluate the effects of ovariectomy (vs sham surgery) and to compare the differences between strains (WKY vs. SHR), data were analyzed by two-way ANOVA followed by Tukey's multiple comparison test. The differences were considered significant when $p<0.05$.

Results

Baseline measurements

The mean values (± SEM) of body weight and baseline PI and SAP obtained over a 30 minute period are shown in Table 1. These values were stable and represent typical resting levels in both SHR and WKY female rats. As expected, SHR-SHAM presented a significantly higher arterial blood pressure and lower pulse interval as compared to WKY-SHAM rats. Ovariectomy did not change PI or SAP of either SHR-OVX or WKY-OVX rats.

Spectral analysis of heart rate and arterial pressure variability

The power spectral densities of PI and SAP variability are shown in Table 2. Notice that ovariectomy did not change the spectral oscillations of cardiovascular variability in WKY-OVX rats. In OVX-SHR, ovariectomy increased LF and decreased the HF component, both expressed in normalized units, and also shifted the sympatho-vagal balance of PI variability toward sympathetic predominance (Table 2).

SAP variability was unaffected by the removal of the ovaries in WKY-OVX rats, whereas SHR-OVX presented a significant increase in total variance and in the VLF component. LF and HF oscillations of SAP variability did not differ between the SHR-OVX and SHR-SHAM groups (Table 2). Fig. 1(A) and (B) illustrates the individual tracings and spectra of SAP variability in all experimental groups.

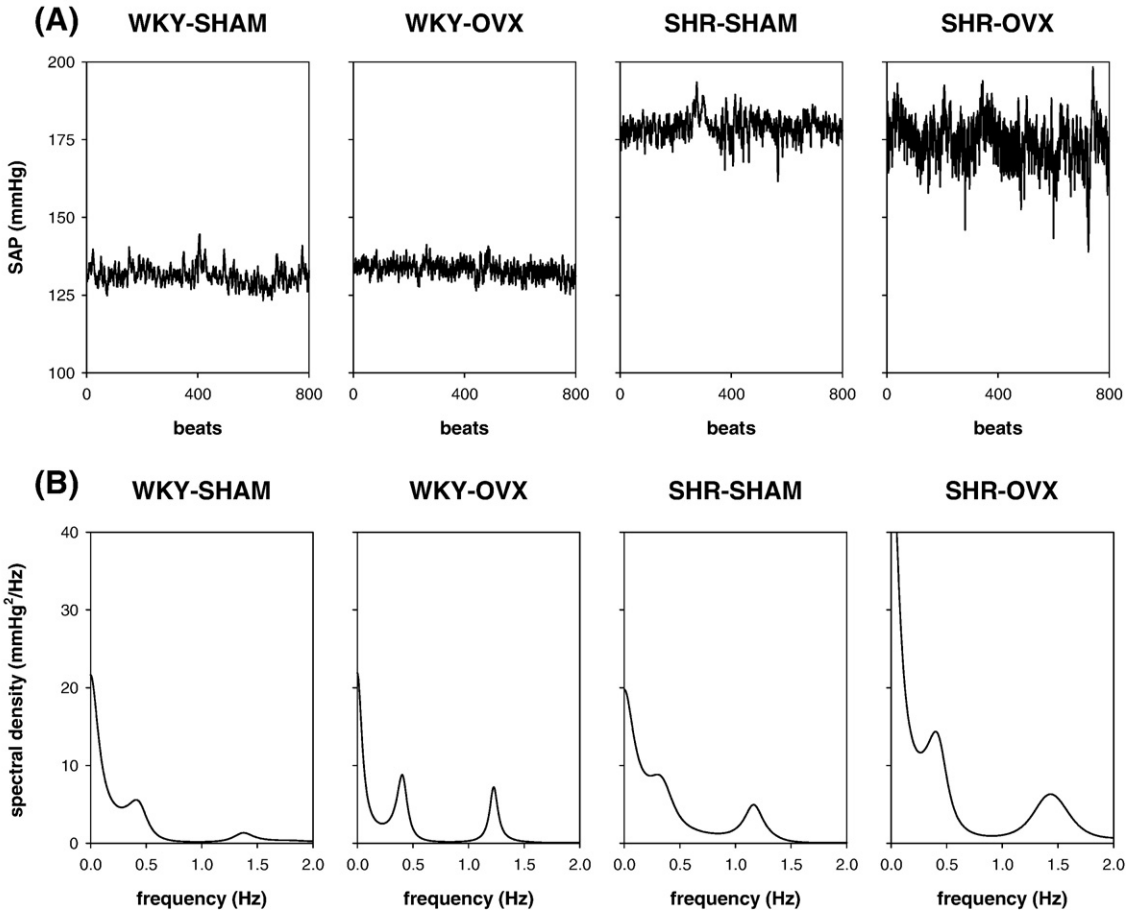

Fig. 1. (A) Time series of systolic arterial pressure (SAP) variability and (B) Power spectra of systolic arterial pressure (SAP) variability of individual rats representative of normotensive WKY and spontaneously hypertensive rats (SHR) submitted to ovariectomy (OVX) or sham surgery (SHAM).

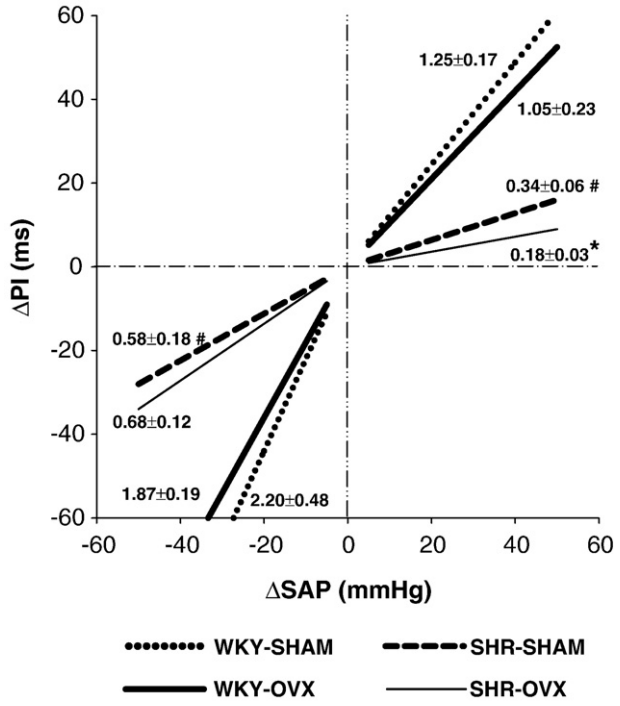

Fig. 2. Reflex control of heart rate in WKY and SHR submitted to ovariectomy (OVX) or sham surgery (SHAM). The lines represent the linear regressions between changes in the pulse interval and systolic arterial pressure. Numbers indicate the slope of each linear regression (baroreflex gain). *$p<0.05$ versus SHR-SHAM; #$p<0.05$ versus respective WKY-SHAM.

Baroreflex sensitivity

Concerning BRS (Fig. 2), reflex tachycardia and reflex bradycardia were unaffected by the removal of the ovaries in WKY-OVX. On the other hand, the reflex bradycardia, but not reflex tachycardia, was reduced by ovariectomy in SHR-OVX (0.18 ± 0.03 ms/mmHg vs. 0.34 ± 0.06 ms/mmHg in SHR-SHAM, $p<0.05$). In addition, spontaneous BRS calculated as the α-index from PI and SAP variability is presented in Table 2. Notice the reduced α-index of SHR-OVX as compared to SHR-SHAM. The α-indexes were not changed in both WKY-SHAM and WKY-OVX rats. All these spontaneous BRS data reinforce those obtained from pharmacological baroreflex procedure.

Discussion

To our knowledge, this is the first study to examine the effect of chronic ovariectomy on cardiovascular variability and baroreflex control in SHR, a widely used rat model of arterial hypertension. Our findings suggest that the lack of ovarian hormones shifts the cardiac sympatho-vagal balance toward sympathetic predominance and worsens the already blunted baroreflex sensitivity in SHR, an outcome that may be related to the significant increase in arterial pressure variability. Extrapolating to human beings, these changes may additionally increase the cardiovascular risk associated with hypertension during estrogen lack.

The present investigation showed that chronic (16 weeks) ovarian hormone deficiency did not affect the basal levels of arterial pressure or heart rate in WKY-OVX and SHR-OVX, suggesting that estrogen does not participate in the long-term maintenance of arterial pressure or heart rate in normotensive WKY or SHR. Despite of some reports using normotensive rats have reported increases of arterial pressure post-ovariectomy (Mercier et al. 2002; Thorin et al. 2003; Irigoyen et al. 2005; Souza et al. 2007), our data match with almost all studies using normotensive rats, which did not show an increase in arterial pressure post-ovariectomy (Takezawa et al. 1994a,b; Lam et al. 2002; Brandin et al. 2003; El-Mas and Abdel-Rahman 1998, 2004; Tezini et al. 2008). In some of these papers, arterial pressure was also evaluated for long term by means of telemetry system, in which manipulation stress effects are absent (Takezawa et al. 1994a,b; Brandin et al. 2003; El-Mas and Abdel-Rahman 2004). Similar findings of no increasing in arterial pressure post-ovariectomy were also observed for hypertensive animals, including SHRs (Hayward et al. 2000; Reckelhoff et al. 2000; Swislocki et al. 2002; Sullivan et al. 2007; Jazbutyte et al. 2008; Martin et al. 2008), even though Ito et al. (2006) have observed an increase in arterial pressure post-ovariectomy in SHR. Taking into consideration the clinical context, interestingly, the arterial blood pressure does not increase during the transitional phase from perimenopause to menopause (Luoto et al. 2000), but rather the increase in blood pressure after menopause takes an average of 5 to 20 years to develop (Burt et al. 1995), suggesting that lack of female hormones may not be the only contributing factor for the elevated blood pressure observed in menopause women.

Although estrogen does not appear to play a role in the long-term maintenance of basal heart rate levels, this hormone seems to be very important in maintaining a relatively normal autonomic balance in the heart, at least in SHR. After long-term ovariectomy, a significant shift of sympatho-vagal balance (expressed as LF/HF ratio) towards sympathetic predominance was observed in ovariectomized SHR. With respect to BRS the data obtained in the present study demonstrate that ovariectomized SHR presented a further attenuation of the already depressed vagal baroreflex control of heart rate. These results suggest that, at least in SHR, ovarian hormones may play a role in the autonomic control of the heart, reducing sympathetic activity and/or increasing parasympathetic drive. Previous studies in the literature provide support to these indirect findings (Lipsitz et al. 1995; Huikuri et al. 1996). It has been shown in the literature that this autonomic imbalance, expressed as change of the sympatho-vagal balance and/or depressed BRS, can be associated with an augmented cardiovascular risk (Malliani et al. 1991; Task Force 1996; La Rovere et al. 2001). Accordingly, it may be hypothesized that these cardiac autonomic changes observed after estrogen deficiency in rats, might explain, at least in part, the increased cardiovascular risk associated, in a clinical context, with the lack of estrogen in women (Lipsitz et al. 1995; Huikuri et al. 1996).

Although estrogen seems to be unimportant in the maintenance of chronic arterial pressure levels, this hormone seems to be crucial, at least in SHR, for the attenuation of arterial pressure variability in the VLF range, as indicated by the significant higher values of total variance and VLF oscillations of SAP in ovariectomized SHR. Interestingly, after ovariectomy, total variance of SAP variability of female SHR reaches similar values when compared to total variance of SAP variability found in male SHR (Dias da Silva et al. 2001), suggesting that the lower arterial pressure variability observed in non-ovariectomized female SHR could be due, at least partially, to the presence of estrogen.

It should be pointed out that VLF fluctuations of AP variability have not been thoroughly studied. There are few reports in the literature (Cerutti et al. 1994) documenting a role for the baroreflex dealing with continuous buffering of VLF oscillations of arterial pressure. In fact, sinoaortic deafferentation in normotensive rats (Cerutti et al. 1994) led to marked increases in variance and in the VLF component of arterial pressure, providing support for a role of the arterial baroreceptors. Taking into consideration the role played by arterial baroreceptors in VLF modulation of arterial pressure variability, the results of the present study strongly suggest that the higher arterial pressure variability observed in female SHR under estrogen deficiency may be at least partially related to further attenuation of baroreceptor function, as indicated by the poor baroreflex control of heart rate in ovariectomized SHR, quantified using pharmacological procedure with vasoactive drugs or using spontaneous oscillations of PI and SAP, as the α-index. In fact, these results are reinforced by some

reports in the literature (Mohamed et al. 1999; Saleh and Connell 1999; Saleh et al. 2000) that describe an effect of central or peripheral administration of exogenous estrogen increasing baroreflex control of heart rate in rats. Even though these reports did not prove a role played by endogenous estrogen controlling peripheral (afferents) or central baroreflex pathways, they strongly suggest that estrogen receptors are present in some point of these pathways and that endogenous estrogens could be important to modulate baroreflex control of circulation, at least, in hypertensive animals.

In addition to the role played by arterial baroreceptors in VLF modulation of arterial pressure variability, other mechanisms has been suggested to explain the VLF oscillations, such as thermoregulation, vasopressin, dark–light cycle, endothelium-derived nitric oxide (NO) and renin–angiotensin system, among others (Akselrod et al. 1985). Since estrogen is able to reduce the expression of angiotensin AT-1 receptors (Nickenig et al. 1998; Fischer et al. 2002) and to increase NO bio-availability as well as to improve endothelial dysfunction (Squadrito et al. 2000; Sader and Celermajer 2002; Tatchum-Talom et al. 2002), we may hypothesize that changes in these vasoactive agents after ovariectomy could also explain, at least partially, the significant increase in the VLF component in ovariectomized SHR. Nevertheless, additional investigation is necessary to strengthen this hypothesis.

In the present study, no effect of ovariectomy on cardiovascular oscillations or BRS was demonstrated in female normotensive WKY rats. This lack of effect of ovariectomy in WKY rats may not be ascribed to an artifact related to natural variations of plasma levels of ovarian hormones during estrus cycle in rats, since we have taken care to record the cardiovascular parameters of all sham-operated animals in a same phase of the estrous cycle, the estrus phase, in which the plasma levels of ovarian hormones are high (Yoshinaga et al. 1969). It is possible that, under normotensive conditions, the influence of estrogen hormones on cardiovascular control is reduced, making it invisible sixteen weeks after ovariectomy. The reasons for that are not known but seem to match with the lack of HRV changes with cyclic variations in estrogens in normotensive women (Leicht et al. 2003). Although no difference in plasma estrogen levels has been documented in SHR and WKY rats (Dubey et al. 2002), it is possible that the influence of estrogen is higher in SHR, playing a counter-regulatory role leading to an attenuation of the deleterious effects of arterial hypertension. Under this condition, a role for estrogen could be more visible after long-term ovariectomy.

Clinical studies using ambulatory arterial pressure monitoring have revealed that hypertensive patients with higher arterial pressure variability have an augmented cardiovascular risk with a marked tendency toward the development of stroke and renal damage (Devereux and Pickering 1991). The present study suggests that, at least partially, the higher arterial pressure variability, if present in postmenopausal female hypertensive patients, may be dependent on estrogen deficiency, and that these patients would benefit by hormonal replacement therapy (Hayward et al. 2000).

One limitation of the present study is that a phytoestrogen-free chow was not used to feed both strains. Therefore, there is a possibility that estrogen levels were not completely depleted (Altavilla et al. 2001; Fang et al. 2001). However, all ovariectomized rats presented permanent diestrous under vaginal smear analysis, indicating a remarkable depletion of estrogen. In addition, it is realized that studies of ovariectomy in animals cannot be held as perfect surrogate of menopause in women, which occurs gradually in association with aging (Hayward et al. 2000). Thus, the extrapolations of these results to postmenopausal hypertensive women are taken into consideration very cautiously. Despite of that, the abrupt decline in hormonal levels in the OVX animal model perfectly simulates clinical settings which necessitate surgical removal of the ovaries. At the end, caution should be also taken to ascribe all the effects of ovariectomy to the lack of estrogen. Further studies, including estrogen and/or progesterone reposition are imperative in order to establish the role played by estrogen and/or progesterone on the arterial pressure variability and cardiac autonomic control. In addition, the use of RAS inhibition and NOS potentiation should be implemented in future experiments to test the hypothesis of participation of RAS over-activity or NOS dysfunction increasing arterial pressure variability after ovariectomy. The results of these experiments could present an important clinical implication, since the use of RAS blockers or augmentation of NOS activity could reduce cardiovascular risk in women with ovarian hormones deprivation.

In summary, the lack of ovarian hormones elicited by ovariectomy induces significant changes in cardiovascular variability, and cardiac baroreflex control, in SHR, which might be deleterious to the cardiovascular system and might contribute to the higher cardiovascular risk in hypertensive postmenopausal women.

Acknowledgements

This study was financially supported by a grant (#CBB-181/02) from Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Brazil. R.M., L.O. and C.H.F.R.A. were supported by fellowships from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil. N.M. and A.P. were supported by a 2005 FIRST Grant from the University of Milan, Italy.

References

Akselrod S, Gordon D, Madwed JB, Snidman DC, Shannon DC, Cohen RJ. Hemodynamic regulation: investigation by spectral analysis. American Journal of Physiology 249, H867–H875, 1985.

Altavilla D, Saitta A, Galeano M, Squadrito G, Marino D, Minutoli L, Calapai G, Deodato B, D'Anna R, Corrado F, Caputi AP, Squadrito F. The phytoestrogen alpha-zearalenol reverses endothelial dysfunction induced by oophorectomy in rats. Laboratory Investigation 81, 125–132, 2001.

Becker RC, Corrao JM. Cardiovascular disease in women. Cardiology 77, 1–140, 1990.

Brandin L, Bergstrom G, Manhem K, Gustafsson H. Oestrogen modulates vascular adrenergic reactivity of the spontaneously hypertensive rat. Journal of Hypertension 21, 1695–1702, 2003.

Burt VL, Whelton P, Roccella EJ, Brown C, Cutler JA, Higgins M, Horan MJ, Labarthe D. Prevalence of hypertension in the US adult population. Results from the Third National Health and Nutrition Examination Survey, 1988–1991. Hypertension 25, 305–313, 1995.

Cerutti C, Barres C, Paultre C. Baroreflex modulation of blood pressure and heart rate variabilities in rats: assessment by spectral analysis. American Journal of Physiology 266, H1993–H2000, 1994.

Dahlberg ST. Gender difference in the risk factors for sudden cardiac death. Cardiology 77, 31–40, 1990.

Dart AM, Du XJ, Kingwell BA. Gender, sex hormones and autonomic nervous control of the cardiovascular system. Cardiovascular Research 53, 678–687, 2002.

Devereux RB, Pickering TG. Relationship between the level, pattern and variability of ambulatory blood pressure and target organ damage in hypertension. Journal of Hypertension 9, S34–S38, 1991.

Dias Da Silva VJ, Cavalcante Viana PC, de Melo Alves R, Salgado HC, Montano N, Fazan Jr R. Antihypertensive action of amiodarone in spontaneously hypertensive rats. Hypertension 38, 597–601, 2001.

Dubey RK, Oparil S, Imthurn B, Jackson EK. Sex hormones and hypertension. Cardiovascular Research 53, 688–708, 2002.

El-Mas MM, Abdel-Rahman AA. Estrogen enhances baroreflex control of heart rate in conscious ovariectomized rats. Canadian Journal of Physiology and Pharmacology 76, 381–386, 1998.

El-Mas MM, Abdel-Rahman AA. Effects of long-term ovariectomy and estrogen replacement on clonidine-evoked reductions in blood pressure and hemodynamic variability. Journal of Cardiovascular Pharmacology 43, 607–615, 2004.

Fang Z, Carlson SH, Chen YF, Oparil S, Wyss JM. Estrogen depletion induces NaCl-sensitive hypertension in female spontaneously hypertensive rats. American Journal of Physiology 281, R1934–R1939, 2001.

Fischer M, Baessler A, Schunkert H. Renin angiotensin system and gender differences in the cardiovascular system. Cardiovascular Research 53, 672–677, 2002.

Hayward CS, Webb CM, Collins P. The roles of gender, the menopause and hormone replacement therapy on cardiovascular function. Cardiovascular Research 46, 28–49, 2000.

Head GA, McCarty R. Vagal and sympathetic components of the heart rate range and gain of the baroreceptor-heart rate reflex in conscious rats. Journal of Autonomic Nervous System 21, 203–213, 1987.

Huikuri HV, Pikkujamsa SM, Airaksinen KE, Ikaheimo MJ, Rantala AO, Kauma H, Lilja M, Kesaniemi YA. Sex-related differences in autonomic modulation of heart rate in middle-aged subjects. Circulation 94, 122–125, 1996.

Irigoyen MC, Paulini J, Flores LJ, Flues K, Bertagnolli M, Moreira ED, Consolim-Colombo F, Belló-Klein A, De Angelis K. Exercise training improves baroreflex sensitivity

associated with oxidative stress reduction in ovariectomized rats. Hypertension 46, 998–1003, 2005.
Ito K, Hirooka Y, Kimura Y, Sagara Y, Sunagawa K. Ovariectomy augments hypertension through rho-kinase activation in the brain stem in female spontaneously hypertensive rats. Hypertension 48, 651–657, 2006.
Jazbutyte V, Arias-Loza PA, Hu K, Widder J, Govindaraj V, von Poser-Klein C, Bauersachs J, Fritzemeier KH, Hegele-Hartung C, Neyses L, Ertl G, Pelzer T. Ligand-dependent activation of ERβ lowers blood pressure and attenuates cardiac hypertrophy in ovariectomized spontaneously hypertensive rats. Cardiovascular Research 77, 774–781, 2008.
La Rovere MT, Pinna GD, Hohnloser SH, Marcus FI, Mortara A, Nohara R, Bigger Jr JT, Camm AJ, Schwartz PJ. ATRAMI Investigators. Baroreflex sensitivity and heart rate variability in the identification of patients at risk for life-threatening arrhythmias: implications for clinical trials. Circulation 103, 2072–2077, 2001.
Lam KK, Hu CT, Ou TY, Yen MH, Chen HI. Effects of oestrogen replacement on steady and pulsatile haemodynamics in ovariectomized rats. British Journal of Pharmacology 136, 811–818, 2002.
Leicht AS, Hirning DA, Allen GD. Heart rate variability and endogenous sex hormones during the menstrual cycle in young women. Experimental Physiology 88, 441–446, 2003.
Lipsitz LA, Connelly CM, Kelley-Gagnon M, Kiely DK, Morin RJ. Effects of chronic estrogen replacement therapy on beat-to-beat blood pressure dynamics in healthy postmenopausal women. Hypertension 26, 711–715, 1995.
Luoto R, Sharrett AR, Schreiner P, Sorlie PD, Arnett D, Ephross S. Blood pressure and menopausal transition: The Atherosclerosis Risk in Communities study (1987–95). Journal of Hypertension 18, 27–33, 2000.
Malliani A, Pagani M, Lombardi F, Cerutti S. Cardiovascular neural regulation explored in the frequency domain. Circulation 84, 482–492, 1991.
Martin DS, Redetzke R, Vogel E, Mark C, Eyster KM. Effect of ovariectomy on blood pressure and venous tone in female spontaneously hypertensive rats. American Journal of Hypertension 21, 983–988, 2008.
Mercier I, Pham-Dang M, Clement R, Gosselin H, Colombo F, Rouleau JL, Calderone A. Elevated mean arterial pressure in the ovariectomized rat was normalized by ET(A) receptor antagonist therapy: Absence of cardiac hypertrophy and fibrosis. British Journal of Pharmacology 136, 685–692, 2002.
Mohamed MK, El-Mas MM, Abdel-Rahman AA. Estrogen enhancement of baroreflex sensitivity is centrally mediated. American Journal of Physiology 276, R1030–R1037, 1999.
Montano N, Ruscone TG, Porta A, Lombardi F, Pagani M, Malliani A. Power spectrum analysis of heart rate variability to assess the changes in sympathovagal balance during graded orthostatic tilt. Circulation 90, 1826–1831, 1994.
Nickenig G, Baumer AT, Grohe C, Kahlert S, Strehlow K, Rosenkranz S, Stablein A, Beckers F, Smits JF, Daemen MJ, Vetter H, Bohm M. Estrogen modulates AT1 receptor gene expression in vitro and in vivo. Circulation 97, 2197–2201, 1998.
Pagani M, Somers V, Furlan R, Dell'Orto S, Conway J, Baselli G, Cerutti S, Sleight P, Malliani A. Changes in autonomic regulation induced by physical training in mild hypertension. Hypertension 12, 600–610, 1988.
Reckelhoff JF, Zhang H, Srivastava K. Gender differences in development of hypertension in spontaneously hypertensive rats: role of the renin-angiotensin system. Hypertension 35, 480–483, 2000.
Rubini R, Porta A, Baselli G, Cerutti S, Paro M. Power spectrum analysis of cardiovascular variability monitored by telemetry in conscious unrestrained rats. Journal of Autonomic Nervous System 45, 181–190, 1993.
Sader MA, Celermajer DS. Endothelial function, vascular reactivity and gender differences in the cardiovascular system. Cardiovascular Research 53, 597–604, 2002.
Saeki Y, Atogami F, Takahashi K, Yoshizawa T. Reflex control of autonomic function induced by posture change during the menstrual cycle. Journal of Autonomic Nervous System 66, 69–74, 1997.
Saleh TM, Connell BJ. Centrally mediated effect of 17-beta-estradiol on parasympathetic tone in male rats. American Journal of Physiology 276, R474–R481, 1999.
Saleh MC, Connell BJ, Saleh TM. Autonomic and cardiovascular reflex responses to central estrogen injection in ovariectomized female rats. Brain Research 879, 105–114, 2000.
Sato N, Miyake S, Akatsu J, Kumashiro M. Power spectral analysis of heart rate variability in healthy young women during the normal menstrual cycle. Psychosomatic Medicine 57, 331–335, 1995.
Souza SB, Flues K, Paulini J, Mostarda C, Rodrigues B, Souza LE, Irigoyen MC, De Angelis K. Role of exercise training in cardiovascular autonomic dysfunction and mortality in diabetic ovariectomized rats. Hypertension 50, 786–791, 2007.
Squadrito F, Altavilla D, Squadrito G, Saitta A, Cucinotta D, Minutoli L, Deodato B, Ferlito M, Campo GM, Bova A, Caputi AP. Genistein supplementation and estrogen replacement therapy improve endothelial dysfunction induced by ovariectomy in rats. Cardiovascular Research 45, 454–462, 2000.
Sullivan JC, Semprun-Prieto L, Boesen EI, Pollock DM, Pollock JS. Sex and sex hormones influence the development of albuminuria and renal macrophage infiltration in spontaneously hypertensive rats. American Journal of Physiology: Regulatory, Integrative and Comparative Physiology 293, R1573–R1579, 2007.
Swislocki A, Burgie ES, Rodnick KJ. Effects of ovariectomy on indices of insulin resistance, hypertension, and cardiac energy metabolism in middle-aged spontaneously hypertensive rats (SHR). Hormones and Metabolism Research 34, 516–522, 2002.
Takezawa H, Hayashi H, Sano H, Ebihara Sa. Estrous-related variations of blood pressure and heart rate in female rats. Frontiers in Medicine and Biological Engineer 6, 131–137, 1994a.
Takezawa H, Hayashi H, Sano H, Saito H, Ebihara Sb. Circadian and estrous cycle-dependent variations in blood pressure and heart rate in female rats. American Journal of Physiology 267, R1250–R1256, 1994b.
Task Force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Circulation 93, 1043–1065, 1996.
Tatchum-Talom R, Martel C, Marette A. Influence of estrogen on aortic stiffness and endothelial function in female rats. American Journal of Physiology: Heart and Circulatory Physiology 282, H491–H498, 2002.
Tezini GC, Silveira LC, Maida KD, Blanco JH, Souza HC. The effect of ovariectomy on cardiac autonomic control in rats submitted to aerobic physical training. Autonomic Neuroscience 143, 5–11, 2008.
Thorin E, Pham-Dang M, Clement R, Mercier I, Calderone A. Hyper-reactivity of cerebral arteries from ovariectomized rats: Therapeutic benefit of tamoxifen. British Journal of Pharmacology 140, 1187–1192, 2003.
Yoshinaga K, Hawkins RA, Stocker JF. Estrogen secretion by the rat ovary in vivo during the estrous cycle and pregnancy. Endocrinology 85, 103–112, 1969.