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Ageing is the main determinant of haemodynamics and autonomic cardiac changes observed in post-menopausal female rats

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ABSTRACT

The aim of this study was to evaluate and compare the effects of early and physiological menopause on cardiac autonomic parameters in aged female rats. To this end, female Wistar rats (22 and 82 weeks old, N = 96) were divided into 4 groups: Young Sham-operated Rats, Aged Sham-operated Rats, Young Ovariectomised (OVX) Rats, and Aged OVX Rats. Young Sham-operated and OVX rats were used as controls. The cardiac autonomic parameters were investigated using different approaches: 1) pharmacological evaluation of the autonomic tonus with methylatropine and propranolol; 2) isolated cardiac contractility with β-adrenergic agonists; and 3) quantification of the mRNA and protein level expression of cardiac β-adrenergic receptors. Among the groups of aged female rats, both the Sham-operated and OVX rats showed higher basal mean arterial pressure and heart rate (HR) values compared to their respective young counterparts. The aged groups also showed a predominance of the sympathetic autonomic component in the determination of HR, whereas the young rats showed a vagal predominance. An assessment of cardiac contractility showed that aged Sham-operated and OVX rats had lower contractile responses following the administration of dobutamine compared to their respective young counterparts. In addition, the aged groups showed higher mRNA and protein expression levels of the β_1-adrenergic receptors. In conclusion, our results show that haemodynamic alterations and impairment of the autonomic parameters were similar between the groups of rats subjected to early and physiological menopause. Moreover, these results seem to be due to the ageing process and not ovarian hormone deprivation.

1. Introduction

Several epidemiological studies have shown that the loss of ovarian function, either spontaneously or surgically induced, before the age of 40, a condition defined as early menopause (Coulam et al., 1986), has been associated with an increased risk of myocardial infarction, stroke and mortality (Jacobsen et al., 1999; Mondul et al., 2005; Rivera et al., 2009). In addition, some authors postulate that this condition, compared to physiological menopause, produces even greater cardiovascular risks (Van der Schouw et al., 1996; Atsma et al., 2006; Lobo, 2007).

Based on such information, ovarian hormones seem to have a protective effect on the cardiovascular system (Miller and Duckles, 2008). Moreover, the early loss of female hormones seems to be a determinant for a higher susceptibility to cardiovascular diseases (CVDs). Therefore, the hypothesis raised is that early surgical removal of the ovaries, when associated with the ageing process, is more devastating for the cardiovascular system than when ovaries are preserved during the ageing process.

The increase in the risk of CVDs with ageing is commonly associated with impairments in the cardiac autonomic control of both men and women, and an evaluation of the cardiac sympathetic–vagal balance is used to predict the cardiovascular risk (Malliani et al., 1991; Task Force, 1996; La Rovere et al., 2001). In this sense, some studies have suggested that female hormones are beneficial for cardiovascular autonomic control (Huikuri et al., 1996; Saleh and Connell, 1999; Saleh et al., 2000; Dart et al., 2002), even for the autonomic impairments resulting from physiological menopause (Lipsitz et al., 1995; Huikuri et al., 1996; Davy et al., 1998; Ribeiro et al., 2001; Neves et al., 2007). However, although the detrimental effects of early menopause on the cardiovascular system have already been addressed in the literature, no study has investigated the implication of early menopause on the autonomic regulation when associated with ageing.

Additionally, changes in other components of the cardiac autonomic control, such as sensitivity and the density of the cardiac β-adrenergic receptors, also deserve attention because cardiac responsiveness to β-adrenergic stimulation is a key factor in the inotropic, chronotropic

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and lusitropic regulation of the myocardium (Brodde, 1991; Brodde and Michel, 1999). Furthermore, studies have shown that in physiological situations, such as hypertension and heart failure, β-adrenergic signalling is impaired, which can compromise the overall functional capacity (Atkins et al., 1995; Nagata et al., 2000; Hamdani and Linke, in press). In this specific case, studies investigating this aspect were conducted using young female rats that were subjected to an ovariectomy without associating the results with the ageing process (Thawornkaiwong et al., 2003; Kam et al., 2004).

Therefore, the objective of the present study was to assess and compare the effects of early and physiological menopause on cardiac autonomic parameters with different approaches, including double pharmacological autonomic blockade, isolated cardiac contractility using β-adrenergic agonists and quantification of mRNA and protein expression of the cardiac β-adrenergic receptors.

2. Materials and methods

2.1. Animals

The research trials were performed on female Wistar rats supplied by the Animal Facility of the School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto. The rats were housed in a room with a strictly controlled temperature (23 ± 2 °C) and a 12-h light/dark cycle with unrestricted access to tap water and standard rat chow (Nuvilab CR-1, Nuvital, Brazil). All of the experimental protocols performed in the current study were approved by the Committee on Animal Research and Ethics of the School of Medicine of Ribeirão Preto, University of São Paulo (Protocol #198/2008).

2.2. Experimental groups

Ninety-six female Wistar rats (10 weeks old) were initially subjected to ovariectomy (OVX) or a sham surgery (Sham-operated) and, after surgical recovery, were divided into four experimental groups (22 and 82 weeks old): Young Sham-operated Rats (N = 24); Aged Sham-operated Rats (N = 24); Young OVX Rats (N = 24); and Aged OVX Rats (N = 24). Young Sham-operated and OVX rats were used as controls.

2.3. Ovariectomy

The rats were anaesthetised with tribromoethanol (250 mg/kg, *i.p.*), and a small abdominal incision was made. The ovaries were located, and a silk thread was tightly tied around the oviduct, including the ovarian blood vessels. The oviduct was sectioned, and the ovary was removed. The contralateral ovary was removed in a similar manner. The skin and muscle walls were then sutured with a silk thread. All of the animals received prophylactic antibiotic therapy following the surgical procedures. The Sham-operated rats underwent the same procedure except for the sectioning of the oviducts and the removal of the ovaries. The rats were housed individually, and a 2-week post-surgical recovery period was allowed. Subsequently, the rats were housed in groups of 3 per cage and were allowed to rest for 10 weeks (young groups) or 70 weeks (aged groups) before the experiments began. During the last 4 weeks, a vaginal smear was collected daily to verify oestrous cycle regularity in the young Sham-operated group and the absence of an oestrous cycle (i.e., permanent diestrous state) in the aged Sham-operated, the young OVX and the aged OVX groups.

2.4. Experimental protocol

2.4.1. Cardiac sympathovagal balance and intrinsic heart rate

At the end of the experimental period, 12 rats per group were anaesthetised with tribromoethanol (250 mg/kg, *i.p.*), and polyethylene catheters were implanted into the right femoral artery and vein. The catheters were tunnelled subcutaneously and exteriorised in the nape. To prevent the blood from clotting, the catheters were filled with heparinised saline solution (500 IU/mL). The rats were then allowed to recover for 24 h prior to the cardiac sympathovagal assessment protocol, which was carried out without anaesthesia.

The assessment of the influence of sympathetic and parasympathetic autonomic tone on the HR was performed by administering propranolol (4 mg/kg, *i.v.*) and methylatropine (5 mg/kg, *i.v.*), respectively. After 60 min of basal HR (bHR) recording, methylatropine was injected into the rats of each group, and the HR was recorded for the following 15 min to assess the effect of vagal blockade on the HR. Propranolol was then injected in the same rats, and the HR was recorded for another 15 min to determine the intrinsic HR (iHR). After 24 h, the methylatropine–propranolol sequence was reversed to assess the effect of sympathetic blockade on HR, following the same recording procedure (15 min each) for each drug, as described previously, to determine the iHR. The data from the methylatropine–propranolol and propranolol–methylatropine sequences were pooled to provide the bHR (before any drugs) and the iHR. In addition, the cardiac sympathovagal index (SVI) was calculated as the ratio between the bHR and the iHR (bHR/ iHR). This index expresses the tonic autonomic balance of the heart, where values above 1 show a predominance of a sympathetic tone, and values below 1 show a predominance of vagal tone (Goldberger, 1999).

2.4.2. Cardiac contractility

Isolation and perfusion of the rat heart: At the end of the experimental period, 6 rats per group were anaesthetised and received heparin (5000 UI/kg, *i.v.*). After a 15-min period, the animals were sacrificed by cervical vessel transection. Following exsanguination, the hearts were quickly excised and perfused at a constant flow of 10 mL/min with Krebs buffer (in mM: NaCl 118.4, KCl 4.7, $CaCl_2$ 2.5, $MgSO_47H_2O$ 1.2, $NaHCO_3$ 25.0, KH_2PO_4 1.2, glucose 11.2 and pyruvic acid 2.0) through a cannula inserted into the aorta. The nutrient solution was continuously gassed with 95% O_2–5% CO_2 (pH 7.4) at a pressure of 80 cm of H_2O and maintained at 37 °C. The coronary perfusion pressure was measured using a pressure transducer (HP-1280 C, Hewlett-Packard). To verify the viability of the preparations, the ventricular contractility was monitored throughout the experiment. Thus, a metal hook coupled to a force transducer (Statham) was placed in the heart apex, and an initial tension of 6 g was applied to the organ. The coronary perfusion pressure and force of contraction were recorded using a polygraph (R 611, Beckman). After a 30-min period of stabilisation, dose–response curves were obtained for dobutamine or salbutamol. Increasing doses of the drugs (1–100 nmol) were administrated in a bolus lasting 15 s through a manually sequential application. The maximum response to dobutamine and salbutamol was induced with 50 nmol, and thus, this dose was selected for the subsequent experiments.

2.4.3. RNA isolation and relative quantification of mRNA levels by reverse transcription with subsequent polymerase chain reaction (RT-PCR)

At the end of the experimental period, 6 rats per group were anaesthetised with tribromoethanol (250 mg/kg, *i.p.*) and, after a 15-min period, were euthanised by decapitation. The hearts were quickly removed and then frozen in liquid nitrogen until RNA extraction processing. The frozen hearts were pulverised and homogenised in Trizol reagent (Invitrogen, Carlsbad, CA). Total RNA was extracted according to the standard protocol recommended by the manufacturer and treated with DNase (Invitrogen). Reactions were performed using the Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen). Briefly, the reaction conditions were as follows: initial (50 °C, 2 min; 95 °C, 10 min), followed by 40 cycles of denaturation (95 °C, 15 s) and annealing/extension (60 °C, 1 min) using an Applied Biosystems 7500 Fast Real-time PCR System. Specific primers were designed based on GenBank sequences and were used to amplify the gene fragments of