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Influence of pre-existing hypertension on neuroendocrine and cardiovascular changes evoked by chronic stress in female rats

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ABSTRACT

This study investigated neuroendocrine, autonomic, and cardiovascular changes evoked by daily exposure to the same type of stressor (homotypic) or different aversive stressor stimuli (heterotypic) in 60-days-old female normotensive Wistar rats and female spontaneously hypertensive rats (SHR). Both strains of rats were exposed for 10 consecutive days to either the homotypic stressor repeated restraint stress (RRS) or the heterotypic stressor chronic unpredictable stress (CUS). As expected, SHR had higher baseline blood pressure values and impaired baroreflex activity in relation to normotensive animals. Besides, SHR presented higher plasma corticosterone levels and decreased thymus weight. Both RRS and CUS increased baseline plasma corticosterone concentration and decreased body weight gain in both normotensive and SHR rats. In addition, both stress protocols caused hypertrophy of adrenal glands in normotensive rats. Regarding the cardiovascular effects, RRS increased basal heart rate in both rat strains, which was mediated by an increase in sympathetic tone to the heart. Besides, RRS increased baroreflex-mediated tachycardia in SHR animals, while CUS increased cardiac parasympathetic activity and pacemaker activity in normotensive rats. Taken together, these results indicate a stress type-specific effect, as identified by a vulnerability of both strains to the deleterious cardiovascular effects evoked by the homotypic stressor and a resilience to the impact of the heterotypic stressor. Vulnerability of hypertensive rats was evidenced by the absence of CUS-evoked adaptive cardiovascular responses and an increase of baroreflex tachycardia in SHR animals subjected to RRS. The somatic and HPA axis changes were overall independent of the chronic stress regimen and pre-existing hypertension.

1. Introduction

Clinical and preclinical studies have indicated that the complications related to stress are determined by characteristics of the aversive stimulus, such as type, duration, frequency, controllability, and intensity (Crestani, 2016; Koolhaas et al., 2011; Steptoe and Kivimäki, 2012). The influence of predictability on animal models has been investigated by comparing the effects of chronic stressors involving daily exposure to the same type of aversive stimuli (i.e., homotypic/predictable) versus different stressors (i.e., heterotypic/unpredictable) (Crestani, 2016). These studies have used the repeated restraint stress (RRS) as a homotypic stressor and the chronic unpredictable stress (CUS) as a heterotypic stressor (Crestani, 2016). Studies comparing the impact of RRS versus CUS have demonstrated that the latter induces more severe changes on somatic parameters (e.g., adrenal hypertrophy and thymic involution), hypothalamic-pituitary-adrenal (HPA) axis activity, and anxiety- and depression-like behaviors (Costa-Ferreira et al., 2016; Haile et al., 2001; Magariños and McEwen, 1995; Marin et al., 2007; Pastor-Ciurana et al., 2014). Differences in impact of CUS and RRS on cardiovascular function are less clear. Indeed, studies comparing these two chronic stressors have reported similar mild hypertension, resting tachycardia, increase in cardiac sympathetic activity, and baroreflex impairment following exposure to either stressor (Costa-Ferreira et al., 2016; Duarte et al., 2015; Vieira et al., 2018).

The impact of stress on physiological and psychological processes is also determined by individual characteristics. In this sense, pre-existing cardiovascular complications have been proposed as a factor affecting the impact of stress. Indeed, previous studies documented that hypertensive and normotensive individuals react differently to stressful events. For instance, hypertensive subjects react more intensely to stress than their normotensive counterparts (Al'Absi et al., 1994; Garafova et al., 2014; Lenders et al., 1989; Matsukawa et al., 1991;

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Palatini et al., 2011). Accordingly, preclinical studies also identified that spontaneously hypertensive rats (SHR) - a widely used hypertension model (Trippodo and Frohlich, 1981) - exhibit exacerbated neuroendocrine and behavioral responses to various aversive stimuli when compared to normotensive rats (Djordjevic et al., 2007; Imaki et al., 1998; Roman et al., 2004; van den Buuse et al., 2001). Sympathetic activation and increase of blood pressure and heart rate (HR) caused by various aversive stimuli are also more pronounced in SHR animals (Casto and Printz, 1990; McCarty, 1983; McDougall et al., 2005, 2000; van den Buuse et al., 2001).

The above-mentioned findings indicate a hyper-responsiveness of physiological and behavioral responses during acute sessions of stress in hypertensive animals and humans. Nevertheless, the impact of hypertension on dysfunctions evoked by chronic stressors is still poorly understood. Previous studies comparing normotensive and SHR rats reported either increased baseline blood pressure selectively in the latter (Bernatova et al., 2010) or absence of effect in both strains (Slezak et al., 2014) following exposure to chronic crowding stress. Besides, increased blood pressure in both normotensive and SHR rats was reported following exposure to colony social stress (Toot et al., 2011). Nevertheless, the influence of hypertension in other cardiovascular-related changes evoked by chronic stress such as autonomic imbalance, resting tachycardia, and baroreflex impairment has never been evaluated. Furthermore, evidence of the impact of either CUS or RRS on cardiovascular function of SHR animals is missing. Finally, although evidence of sex-related differences in cardiovascular function (Dubey, 2002) and cardiovascular and neuroendocrine responses to acute stressors (Anishchenko et al., 2007; Eikelis and Van Den Buuse, 2000; Kudielka and Kirschbaum, 2005), the majority of experimental studies have investigated the impact of chronic stressors in male animals. Therefore, our purpose in the present study was to investigate neuroendocrine, autonomic, and cardiovascular changes caused by the exposure to either RRS or CUS in female SHR and normotensive rats.

2. Material and methods

2.1. Animals

Thirty-three female normotensive Wistar rats (11/experimental group) and twenty-six female SHR rats (9 control, 10 RRS, and 7 CUS) 60-days-old were used in the present study. Animals were obtained from the animal breeding facility of the Institute of Biomedical Science/University of São Paulo (São Paulo, SP, Brazil). All animals were housed in collective plastic cages (41 × 34 x 16 cm) (3–4 rats/cage) in a temperature-controlled room at 24 °C in the Animal Facility of the Laboratory of Pharmacology-UNESP. They were kept under a 12:12 h light-dark cycle (lights on between 7:00 h and 19:00 h) with free access to water and standard laboratory food. Housing conditions and experimental procedures were carried out following protocols approved by the Ethical Committee for Use of Animal of the School of Pharmaceutical Sciences/São Paulo State University, which complies with Brazilian and international guidelines for animal use and welfare.

2.2. Chronic stress regimens

The chronic stress regimens were based in protocols previously described by our group (Duarte et al., 2015; Vieira et al., 2018). Therefore, RRS was chosen as a homotypic stressor, whereas CUS was used as a heterotypic stressor. The animals of RRS group were restrained in opaque plastic cylinders (15 cm length and 5.5 cm internal diameter) for 1 h daily starting at 10:00 a.m. for 10 consecutive days. The CUS protocol consisted of exposure to different stressors in a variable schedule for 10 consecutive days, accordingly to protocol described in Duarte et al (2015). The stressors used in the CUS included: 1) restraint stress (60 min); 2) humid sawdust (overnight or all day); 3) cold (4 °C) or room temperature isolation housing; 4) food/water deprivation (overnight); 5) swim stress (4 min); 6) lights on overnight; and 7) lights off during day (120–180 min). All stress sessions were performed in an adjacent room to the animal facility. The RRS and CUS started simultaneously, and during this period animals of control groups were left undisturbed, except for cleaning the cages and body weight measurements, in the animal facility.

2.3. Surgical preparation

Animals were anesthetized with tribromoethanol (250 mg/kg, i.p.) and a polyethylene cannula (a 4 cm segment of PE-10 heat-bound to a 13 cm segment of PE-50) (Clay Adams, Parsippany, NJ, USA) filled with a solution of heparin (50 UI/ml, Hepamax-S®, Blausiegel, Cotia, SP, Brazil) diluted in saline (0.9% NaCl) was implanted into the abdominal aorta through the femoral artery for cardiovascular recording. A second cannula filled with heparin solution was inserted into the femoral vein for the infusion of drugs. Both catheters were tunneled under the skin and exteriorized on the animal's dorsum. After the surgery, rats received a poly-antibiotic formulation containing streptomycin and penicillin (560 mg/ml/kg, i.m.) to prevent infection, and were treated with the non-steroidal anti-inflammatory drug flunixin meglumine (0.5 mg/ml/kg, s.c.) for postoperative analgesia.

2.4. Measurement of cardiovascular parameters

The cannula implanted into the femoral artery was connected to a pressure transducer (DPT100, Utah Medical Products Inc., Midvale, UT, USA). Pulsatile arterial pressure (PAP) was recorded using an amplifier (Quad Bridge Amp, ML224, ADInstruments, NSW, Australia) and an acquisition board (PowerLab 4/30, ML866/P, ADInstruments, NSW, Australia) connected to a personal computer. Mean (MAP), systolic (SAP), and diastolic (DAP) arterial pressure; as well as HR values were derived from PAP recordings.

2.5. Assessment of cardiac autonomic activity and intrinsic HR

Cardiac autonomic activity and intrinsic HR were assessed by intravenously administrating the muscarinic receptor antagonist methylatropine (3 mg/ml/kg) and the β-adrenoceptor antagonist propranolol (4 mg/ml/kg) (Almeida et al., 2015; Duarte et al., 2015; Vieira et al., 2018). The protocol was performed on two separate days. On the first day, animals in all experimental groups received intravenous administration of methylatropine and propranolol in a random order. The interval of treatment between the drugs was 10 min. Twenty-four hours later, animals were treated with methylatropine and propranolol in the opposite sequence to that performed on the first day.

The parasympathetic activity was determined by analyzing the change in basal HR caused by methylatropine, while the sympathetic activity was obtained from the HR response evoked by propranolol treatment. The intrinsic HR was obtained after combined treatment with propranolol and methylatropine on first and second days, and a mean of the two values was calculated for each animal.

2.6. Assessment of baroreflex activity

Baroreflex function was evaluated using the classical pharmacological approach. For this, intravenous infusion of the α_1-adrenoceptor agonist phenylephrine (70 μg/ml at 0.4 ml/min/kg) and the nitric oxide donor sodium nitroprusside (100 μg/ml at 0.8 ml/min/kg) was performed using an infusion pump (K.D. Scientific, Holliston, MA, USA) (Crestani et al., 2010; Engi et al., 2016). Phenylephrine evoked incremental pressor response, whereas sodium nitroprusside caused incremental depressor effects. Infusions of vasoactive drugs were randomized, and the second treatment was not realized before cardiovascular parameters returned to control values (interval between infusions was approximately 5 min). Infusions lasted for 20–30 s, resulting in the