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Immediate and long-term effects of psychological stress during adolescence in cardiovascular function: Comparison of homotypic vs heterotypic stress regimens

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ABSTRACT

Adolescence has been proposed as an ontogenic period of vulnerability to stress. Nevertheless, the impact of stressful events during adolescence in cardiovascular activity is poorly understood. Therefore, the purpose of this study was to investigate the immediate and long-lasting effects of exposure to stressful events during adolescence in cardiovascular function of rats. To this end, we compared the impact of 10-days exposure to two chronic stress protocols: the repeated restraint stress (RRS, homotypic) and chronic variable stress (CVS, heterotypic). Independent groups of animals were tested 24 h (immediate) or three weeks (long-lasting) following completion of stress period. Exposure to CVS, but not RRS, during adolescence increased basal HR values without affecting arterial pressure, which was followed by augmented power of oscillatory component at low frequency (sympathetic-related) of the pulse interval (PI). RRS enhanced variance of the PI with an increase in the power of both low and high (parasympathetic-related) frequency components. RRS also increased the baroreflex gain. Neither RRS nor CVS affected systolic arterial pressure variability. The RRS-evoked changes in PI variability were long-lasting and persisted into adulthood while all alterations evoked by the CVS were reversed in adulthood. These findings indicate a stress type-specific influence in immediate and long-term effects of stress during adolescence in cardiovascular function. While immediate changes in cardiovascular function were mainly observed following CVS, long-lasting autonomic consequences in adulthood were observed only in animals exposed to RRS during adolescence.

1. Introduction

Several lines of evidence correlate psychosocial factors with the pathogenesis of cardiovascular diseases (Rozanski et al., 1999; Steptoe and Kivimaki, 2012). For instance, clinical and preclinical studies have shown a role of factors such as environmental and social stress, anxiety and mood states, and personality traits (e.g., anger and aggressiveness) in etiology and progression of several cardiovascular dysfunctions (Ford et al., 1998; Grippo and Johnson, 2009; Kawachi et al., 1994; Pickering, 1990; Rozanski et al., 2005; Sgoifo et al., 2014). Although the relevance of the topic, the mechanisms and factors explaining the association between psychosocial factors and cardiovascular diseases are still poorly understood.

Adolescence is a period of continuous development of the neural circuits involved in the stress responses (Andersen, 2003; Casey et al., 2008), which can affect how adolescents cope with stress. Indeed, studies in rodents have shown that somatic, behavioral, and neuroendocrine changes induced by chronic exposure to stress are more pronounced in adolescent than adult animals (Doremus-Fitzwater et al., 2009; Jankord et al., 2011; Stone and Quartermain, 1997). Thus, adolescence is proposed to be an ontogenic period of higher vulnerability to stress (Andersen and Teicher, 2008; Dahl, 2004). Nevertheless, the impact of stressful events during adolescence in cardiovascular activity is poorly understood (Maslova et al., 2002; Porter et al., 2004). Furthermore, despite evidence that stress during adolescence evokes long-lasting behavioral changes and neuroplasticity (Buwalda et al., 2011), the cardiovascular

Abbreviations: CVS, chronic variable stress; DAP, diastolic arterial pressure; HF, variability at high frequency; HR, heart rate; LF, variability at low frequency; MAP, mean arterial pressure; PI, pulse interval; RRS, repeated restraint stress; SAP, systolic arterial pressure.

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consequences in adulthood of emotional stress exposure during adolescence are still unknown.

Characteristics of the aversive stimulus (e.g., chronicity, predictability, and severity) may also influence the impact of stress on physiological functions. Preclinical studies have compared the impact of chronic stress protocols involving daily exposure to the same stressor (i.e., homotypic) or different stressors (i.e., heterotypic) (Flak et al., 2012; Kopp et al., 2013; Magarinos and McEwen, 1995). The chronic variable stress (CVS) is a heterotypic stressor widely used to investigate physiological, behavioral and neural consequences of the exposure to stress in rodents (Grippo, 2009; Herman, 2013; Willner, 2005). It has been demonstrated that CVS, relative to RRS, causes a more severe impact in terms of somatic parameters (e.g., adrenal hypertrophy and thymic involution) and neuroendocrine function (Flak et al., 2012; Kopp et al., 2013; Magarinos and McEwen, 1995; Marin et al., 2007). Also, neuroplasticity and behavioral changes induced by chronic stress seems to be related to the stress regimen (Flak et al., 2012; Kopp et al., 2013; Marin et al., 2007). Cardiovascular changes, autonomic unbalance, and alterations in reflex cardiac and sympathetic nerve activity responses to blood pressure changes (i.e., baroreflex activity) have also been reported following exposure to either CVS or homotypic stressors (Conti et al., 2001; Daubert et al., 2012; Grippo et al., 2008). However, so far, no study compared the impact of homotypic vs heterotypic chronic stress regimens in cardiovascular function.

Altogether, above evidence indicate the adolescence as a period of vulnerability to stress. However, the impact of stressful events during adolescence in cardiovascular activity, including evaluation of possible differences among stress paradigms, has never been investigated. Therefore, our purpose in the present study was to investigate the immediate and long-lasting cardiovascular consequences of the exposure to homotypic and heterotypic chronic stress regimens during adolescence.

2. Material and methods

2.1. Animals

Adolescence has been defined in rodents as the ontogenic period from postnatal day 28 to 42 (Spear, 2000), although some authors have extended this period until the postnatal day 59 (Eiland and Romeo, 2013; Tirelli et al., 2003). During this developmental period, the animals present adolescent-typical neurobehavioral characteristics (McCormick et al., 2010; Spear, 2000). Therefore, 28-days-old male Wistar rats were used in the present study for all experiments. Animals were obtained from the animal breeding facility of the Univ. Estadual Paulista—UNESP (Botucatu, SP, Brazil) and were housed in collective plastic cages (3–4 rats/cage) in a temperature-controlled room at 24 °C in the Animal Facility of the Laboratory of Pharmacology, School of Pharmaceutical Sciences, Univ. Estadual Paulista—UNESP. They were kept under a 12:12 h light–dark cycle (lights on between 7:00 a.m. and 7:00 p.m.) with free access to water and standard laboratory food. Housing conditions and experimental procedures were carried out following protocols approved by Ethical Committee for Use of Animal of the School of Pharmaceutical Sciences/UNESP, which complies with the 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 (Cruz et al., 2012; Marin et al., 2007). Therefore, repeated restraint stress (RRS) was chosen as a homotypic stressor while CVS was used as a heterotypic stress regimen. The animals of RRS group were restrained in opaque plastic cylinders for 1 h daily starting at 9:00 a.m. for 10 consecutive days. The CVS protocol consisted of exposure twice-daily to randomly assigned stressors for 10 consecutive days, according to protocol previously described (Cruz et al., 2012; Marin et al., 2007). The stressors were forced swim (3–4 min), restraint stress (60 min), lights on overnight, lights off during the light period of the light/dark cycle (2–3 h), humid sawdust (overnight or all day), cold stress, food and water deprivation overnight, and social isolation overnight. All the stress sessions were performed in an adjacent room to the animal facility. The RRS and CVS started simultaneously, and during this period animals of the control groups were left undisturbed, except for cleaning the cages, in the animal facility.

2.3. Surgical preparation

Animals were anesthetized with tribromoethanol (250 mg/kg, i.p.) and a catheter (a 4 cm segment of PE-10 heat-bound to a 13 cm segment of PE-50) (Clay Adams, Parsippany, NJ, USA) was inserted into the abdominal aorta through the femoral artery for cardiovascular recording. Catheter was tunneled under the skin and exteriorized on the animal's dorsum. After the surgery, rats were treated with a poly-antibiotic formulation (0.27 mg/kg, i.m.; Pentabiotico®; Fort-Dodge, Brazil) to prevent infection, and received the non-steroidal anti-inflammatory drug flunixine meglumine (0.025 mg/kg, i.m.; Banamine®, Schering-Plough, Brazil) for postoperative analgesia.

2.4. Measurement of cardiovascular parameters

Animals were allowed 60 min to adapt to experimental room conditions, such as sound and illumination, before starting cardiovascular recording. The experimental room was temperature controlled (24 °C) and was acoustically isolated from the other rooms. For the recording, the arterial cannula was connected to a pressure transducer (DPT100, Utah Medical Products Inc., Midvale, UT, USA). Pulsatile arterial pressure 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 arterial pressure (MAP), systolic arterial pressure (SAP), diastolic arterial pressure (DAP), and heart rate (HR) values were derived from pulsatile arterial pressure recordings.

2.5. Power spectral analysis of systolic arterial pressure and pulse interval

Power spectral analysis of SAP and pulse interval (PI) was employed to analyze autonomic activity controlling blood vessels and the heart, respectively. For this, beat-to-beat time series of SAP and PI were extracted from the pulsatile arterial pressure signal. Using fast Fourier transform spectral analysis (Software Cardioseries v2.4, available on https://www.sites.google.com/site/cardioseries/home), the overall variability of these series was calculated in the time and frequency domain. The time domain analysis consisted of calculating the variance in the entire time series. For frequency domain analysis, the power of oscillatory components obtained was quantified in two frequency bands: low frequency (LF, 0.20–0.75 Hz) and high frequency (HF, 0.75–3.0 Hz) (Malliani et al., 1991). Oscillations lower than 0.20 Hz were not quantified.

Oscillations of arterial pressure and PI at LF range are representative of the modulatory effects of sympathetic activity controlling vascular tonus and heart activity, respectively; whereas oscillations of PI at HF range are associated with a parasympathetic modulation of the heart (Malliani et al., 1991).