Full length article

Gonadal hormone receptors underlie the resistance of female rats to inflammatory and cardiovascular complications of endotoxemia

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ABSTRACT

The male gender is more vulnerable to immunological complications of sepsis. Here, we tested the hypotheses that female rats are protected against endotoxemia-evoked hypotension and cardiac autonomic dysfunction, and that gonadal hormone receptors account for such protection. Changes in blood pressure, heart rate, and cardiac sympathovagal balance caused by i.v. lipopolysaccharide (LPS) were determined. In male rats, LPS elevated serum TNFα together with falls in blood pressure and rises in heart rate. The spectral index of cardiac sympathovagal balance (low-frequency/high-frequency ratio, LF/HF) was reduced by LPS, suggesting an enhanced parasympathetic dominance. Remarkably, none of these LPS effects was evident in female rats. We also report that pretreatment of female rats with fulvestrant (nonselective estrogen receptor blocker), PHTPP (estrogen receptor β blocker), or mifepristone (progesterone receptor blocker) uncovered clear in

1. Introduction

Sepsis is a fatal clinical condition characterized by a generalized inflammatory response, serious cardiovascular complications, and multiple organ dysfunction (Angus et al., 2001; Gotts and Matthy, 2016). Our previous studies (Sallam et al., 2016a, 2016b) and others (Hom et al., 1995; Zila et al., 2015) have shown that endotoxemia causes hypotension, tachycardia and cardiac autonomic dysfunction in female rats. These deleterious effects could be attributed to the released inflammatory cytokines, e.g. TNF-α, IL-1, and IL-6, which cause mitochondrial injury, alter calcium homeostasis, and disrupt the autonomic neural control (Flierl et al., 2008).

The immune response to sepsis is sexually dimorphic, with females exhibiting better clinical outcomes than age-matched males (Saia et al., 2015; Klein and Flanagan, 2016). This is supported by experimental studies which demonstrated sex-specific expression of pro- and anti-inflammatory cytokines in response to endotoxemia (Zellweger et al., 1997). Losonczy et al. (2000) demonstrated a preferential blood pressure lowering effect for LPS in anesthetized male, but not female, rats. Moreover, echocardiographic studies reported by Chen et al. (2014) revealed more pronounced signs of cardiac dysfunction, e.g. reduced ejection fraction and fractional shortening, in male compared with female mice.

Male and female sex hormone receptors have been identified on immune cells suggesting direct modulatory effects for reproductive hormones on the immune response (Cristofaro et al., 2006). Estrogen receptors are expressed in leukocytes (Gulshan et al., 1990) and can alter their function (Barañao et al., 1991) and reduce synthesis of inflammatory mediators during inflammatory conditions as endotoxemia (Saia et al., 2011). In addition, estrogen acts through estrogen receptor β receptors to protect against TNF-α-induced inflammation (Xing et al., 2007). By contrast, androgens suppress the immune response via decreasing immunoglobulin cytokine production and inhibition of lymphocyte proliferation (Angle et al., 2000). Further, testosterone

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depletion elicits beneficial effects on immune responses after infectious diseases and sepsis (Angele et al., 1997; Angele and Chaudry, 2005). The question whether hemodynamic and autonomic imbalances induced by endotoxemia are sex-related and could be modulated by sex hormones has not been investigated.

While gender differences in the cardiovascular influences of endotoxemia have been noted, the role of sex hormones and their receptors in these settings remains largely elusive. Thus, the prime objective of the current study was test the hypothesis that gonadal hormone receptors account for the sex-related differences in endotoxic manifestations of inflammation, hypotension and cardiac autonomic dysfunction. Experiments were undertaken in conscious age-matched male and female rats to evaluate the inflammatory and cardiovascular effects of endotoxemia in the absence and presence of pharmacologic antagonists of gonadal hormone receptors. A dose of 10 mg/kg of LPS was utilized to induce endotoxemia as in our previous studies (Sallam et al., 2016a, 2016b). The cardiac autonomic activity was assessed by frequency domain analysis of heart rate variability and the subsequent division of the spectrum into predefined components that reflect cardiac sympathetic and parasympathetic activities (Draghici and Taylor, 2016). A blunted heart rate variability is gaining importance as a prognostic tool of cardiovascular disease and mortality (de Castilho et al., 2017).

2. Materials and methods

2.1. Animals

Age-matched male and female Wistar rats (180–220 g, 11–12 weeks old, Faculty of Pharmacy animal facility, Alexandria University, Alexandria, Egypt) were used in this study. All experiments were performed in strict accordance with the guidelines of the animal care and use of the Faculty of Pharmacy, Alexandria University, Egypt (ACUC Project # 28/2014).

2.2. Intravascular cannulation

The methods used for intravascular cannulation and blood pressure measurement were described in our previous studies (Abdel-Rahman et al., 1992; El-Mas et al., 1994, 1997; El-Mas and Abdel-Rahman, 1995). Experiments were carried out 2 days later in conscious rats.

2.3. Frequency-domain analysis of heart rate variability

Spectral fluctuations in heart rate variability provide quantitative estimates of cardiac sympathetic and vagal controls. Heart rate variability was analyzed in the frequency domain using FFT algorithms of R-data series (Stein et al., 1994; El-Mas and Abdel-Rahman, 2007). The R-R intervals were computed by the LabChart Pro software where beats maxima were detected from the derivative of the blood pressure trace. Traces were visually checked for any erroneous beats and ectopic beats were excluded. FFT algorithm was used for direct transformation of data points into power spectral density graphs. Spectra were integrated into 2 specific frequency bands, low-frequency (LF) (0.05–0.75 Hz) and high-frequency (HF) (0.05–0.75 Hz) bands, and expressed in normalized units (LFnu and HFnu). The latter correlate with the cardiac sympathetic and parasympathetic activity, respectively. The LF/HF ratio was computed and taken as a measure of cardiac sympathovagal balance. Spectral data were estimated before (baseline) and at 15-min intervals after LPS treatments. For each time point, 5-min values of each variable were averaged.

2.4. Measurement of serum estrogen, progesterone and TNF-α

Blood samples (1 ml) were withdrawn from the arterial lines of female rats at the end of the experiment for gonadal hormone determination. In all groups, a blood sample (0.5 ml) was withdrawn from the arterial line of each rat 1 h post-LPS for determination of serum TNF-α. Blood was kept to coagulate for 15 min and then centrifuged at 1200g for 10 min. The supernatant (serum) was aspirated and stored at −80°C until used. TNF-α was determined by Rat TNF alpha Platinum ELISA kit (Bender® MedSystems GmbH, Vienna, Austria) as instructed by the manufacturer. Plasma estradiol levels were measured by the radioimmunoassay (COAT-A-COUNT®, Diagnostic Products Corporation, LA, USA) as described in our previous studies (El-Mas et al., 2011). Plasma progesterone was determined by electrochemiluminescence immunoassay (Roche® Diagnostics GmbH, Mannheim, Germany).

2.5. Protocols and experimental groups

2.5.1. Gender differences in hemodynamic and autonomic effects of endotoxemia

Four groups of conscious rats (2 males and 2 females, n = 7 each), pre-instrumented 48 h earlier with femoral indwelling catheters, were used to assess gender differences in hemodynamic and cardiac autonomic effects of LPS. On the experiment day, a period of at least 30 min was allowed for hemodynamic stabilization. Each rat then received a single i.v. injection of LPS (10 mg/kg) or an equal volume of saline after which blood pressure and heart rate were monitored for 3 h.

2.5.2. Role of gonadal hormone receptors in resistance of female rats to endotoxic manifestations

As the results of the preceding experiment showed that the classic cardiovascular effects of endotoxemia (hypotension, tachycardia, and autonomic dysfunction) appeared in male but not female rats, pharmacologic studies were performed to investigate whether the presence of functional gonadal hormone receptors account for the lack of LPS effects in the female population. Five groups of female rats (n = 7 each) were used to assess the cardiovascular effects of LPS after pretreatment, 30 min earlier, with one of the following: (i) fulvestrant (nonselective estrogen receptor blocker), 5 mg/kg i.v. (Fouda et al., 2015) (ii) MPP (selective estrogen receptor α blocker, 200 μg/kg i.v., (Labouesse et al., 2015) (iii) PHTPP (selective estrogen receptor β blocker, 200 μg/kg i.v., (Dumasia et al., 2015) (iv) mifepristone (progesterone receptor blocker, 10 mg/kg s.c., (Gohar et al., 2013), or (v) formestane (aromatase inhibitor, 15 mg/kg/day for 4 days s.c., (Martinez-Mota et al., 2008). Hemodynamic monitoring continued for 3 h after LPS administration. Changes in MAP, heart rate, and frequency domain indices of heart rate variability (total power, LFnu, HFnu, LF/HF ratio) were computed as described earlier at 15 min intervals using a computerized data acquisition system with LabChart-7 pro software (AD Instruments, Australia).

Because the current findings showed that the administration of LPS to fulvestrant-pretreated female rats caused hypotension and cardiac autonomic dysfunction, we tested whether these LPS effects could be mitigated upon concurrent pharmacologic TNFα inhibition by i.v. pentoxifylline (3 mg/kg, Sallam et al., 2016b). For this purpose, one group of 6 female rats was used and treated consecutively with fulvestrant, pentoxifylline, and LPS. Hemodynamic monitoring continued for 3 h after LPS administration.

2.5.3. Effect of androgen receptor blockade or estrogen supplementation on LPS responses in male rats

Two groups of male rats were used to determine if the LPS effects would be altered by prior treatment, 30 min earlier, with s.c. flutamide (androgen receptor blocker, 10 mg/kg; Calderon Guzman et al., 2011) or i.v. estrogen (0.01 mg/kg; Saleh and Connell, 2000).

2.6. Drugs

Ketamine (Alfasan, Woerden, Holland), xylazine (ADWIA Co. S.A.E.,