European Journal of Pharmacology 797 (2017) 143–152

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Cyclosporine counteracts endotoxemia-evoked reductions in blood pressure and cardiac autonomic dysfunction via central sGC/MAPKs signaling in rats

Marwa Y. Sallam, Sahar M. El-Gowilly, Abdel-Galil A. Abdel-Galil, Mahmoud M. El-Mas*

Department of Pharmacology and Toxicology, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt

ARTICLE INFO

Keywords:
Cyclosporine
Endotoxemia
Hypotension
Heart rate variability
Mitogen activated protein kinases
Inflammatory mediators

ABSTRACT

The immunosuppressant drug cyclosporine A (CSA) improves survivability in endotoxemia and offsets associated loss in vascular reactivity and hypotension. We tested the hypothesis that central phosphoinositide-3-kinase (PI3K)/soluble guanylate cyclase (sGC)/mitogen activated protein kinases (MAPKs) cascade modulates the CSA counteraction of endotoxic hypotension and cardiac autonomic dysfunction. The effects of pharmacologic inhibition of these molecular substrates in central pools on CSA interaction with cardiovascular responses evoked by lipopolysaccharide (LPS) were evaluated in conscious rats. CSA (10 mg/kg) reversed the LPS-evoked (i) hypotension and tachycardia, (ii) decreases in time and spectral measures of heart rate variability (HRV), and (iii) increases in serum TNFα and IL-6. These CSA effects disappeared after intracisternal (i.c.) administration of ODQ (sGC inhibitor) but not wortmannin (PI3K inhibitor). When used alone, ODQ or wortmannin abolished the LPS-evoked hypotension and tachycardia, but had no effect on the concomitant reductions in HRV. We also report that the reversal by CSA of LPS hypotension disappeared after treatment with i.c SB203580 ($MAPK_{p38}$ inhibitor) or PD98059 ($MAPK_{ERK}$ inhibitor), in contrast to little effect for SP600125 ($MAPK_{JNK}$ inhibitor). Alternatively, the CSA amelioration of LPS-evoked reductions in HRV was abolished in presence of SP600125 or PD98059, but not SB203580. The single exposure to SP600125 reduced the decreases in blood pressure, but not HRV, caused by LPS whereas SB203580 produced the exact opposite effects. Together, while central sGC/MAPKs circuits modulate the CSA counteraction of endotoxic manifestations, the recruitment of individual MAPKs into this interaction depends on the nature of the cardiovascular response.

1. Introduction

Endotoxemia is the most frequent cause of death in hospitalized patients (Deutschman and Tracey, 2014). It comprises a systemic inflammatory response to bacterial infection that is characterized by hypotension, reduced HRV, cardiac autonomic dysfunction, multiple organ failure, and death (Drosatos et al., 2014; Sallam et al., 2016b). The most common cause of endotoxic shock is the exposure to lipopolysaccharides (LPS), the structural component of a Gram-negative bacterial membrane (Liu et al., 2012). The pathophysiology of endotoxemia involves the formation of inflammatory mediators such as the tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), IL-2, and IL-6. This primary event is followed by the release of secondary inflammatory mediators such as arachidonic acid metabolites, nitric oxide, and platelet activating factor (Filiz et al., 2010). The inflammatory response caused by systemic LPS is paralleled, and possibly triggered by, the upregulation of $MAPK_{p38}$, and $MAPK_{ERK}$ in the carotid chemoreceptor afferent pathway (Fernandez et al., 2011). In a more recent study, we reported that central pools of $MAPK_{JNK}$ and $MAPK_{p38}$ are pivotal for the elicitation of the hypotensive and reduced cardiac autonomic activity in endotoxic rats, respectively (Sallam et al., 2016a). The pharmacological treatment of endotoxemia is diverse and includes antimicrobial agents, vasoactive drugs, and fluid management (Girbes et al., 2008).

CSA is a potent immunosuppressant that has formed the pharmacologic cornerstone for solid organ transplantation (Du et al., 2008). CSA produces several modulatory effects on vascular and immune systems that might be therapeutically useful in acute endotoxemia. This includes the capacity of CSA to increase vascular tone and BP (Staehr et al., 2013; El-Mas et al., 2012), decrease vascular expression of iNOS (Obias et al., 2006; Staehr et al., 2013), and reduce NO production in macrophages exposed to inflammatory stimuli (Hamalainen et al., 2009). Evidence suggests important role for MAPKs signaling in CSA actions. For instance, CSA enhances the migration of human trophoblasts through promoting $MAPK_{ERK}$-mediated NF-κB activation (Wang et al., 2013). Furthermore, CSA upregulates vascular ET_B receptors

* Corresponding author.
E-mail address: mahelm@hotmail.com (M.M. El-Mas).

http://dx.doi.org/10.1016/j.ejphar.2017.01.023
Received 20 December 2016; Received in revised form 13 January 2017; Accepted 19 January 2017
Available online 20 January 2017

through the activation of $MAPK_{ERK}$, $MAPK_{p38}$, and NF-kB pathways (Zheng et al., 2015).

Although the involvement of MAPKs in the biological effects of endotoxemia (Fernandez et al., 2011; Sallam et al., 2016a) or CSA (Wang et al., 2013) has been documented, it is not clear whether central pathways of MAPKs and their upstream effectors (sGC and PI3K) are pivotal for the counteraction by CSA of the cardiovascular and autonomic consequences of endotoxemia. The current study reports on the effects of intracisternal administration of selective pharmacologic inhibition of molecular targets of the MAPKs/PI3K/sGC pathway on the LPS-CSA cardiovascular interaction in conscious freely moving rats pre-instrumented with femoral and intracisternal indwelling catheters. Moreover, changes caused in time- and frequency-domain analyses of HRV were taken as measures of cardiac autonomic activity (Stein et al., 1994; Sgoifo et al., 1997).

2. Materials and methods

Adult male Wistar rats (180–230 g; Faculty of Pharmacy animal facility, Alexandria University, Alexandria, Egypt) were used in this study. All experiments were performed in strict accordance with institutional animal care and use guidelines (ACUC project No. 2014/28).

2.1. Intracisternal cannulation (i.c.)

A stainless steel guide cannula (23 G; Small Parts, Miami, FL, USA) was implanted into the cisterna magna under thiopental anesthesia (50 mg/kg i.p.) 5 days before starting the experiment (i.e. 3 days before intravascular cannulation) (El-Mas et al., 2009, 2012). The cannula was secured in place with dental acrylic cement (Glass Ionomer, Hangzhou, China) and was considered patent when spontaneous outflow of cerebrospinal fluid was observed and by gross post-mortem histological verification following injection of 5 µl of fast green dye (EM Science; Cherry Hill, NJ, USA). After i.c. cannulation, rats were housed individually.

2.2. Intravascular cannulation

As described in our previous studies (El-Mas and Abdel-Rahman, 1995; El-Mas et al., 1997), rats were anesthetized with thiopental (50 mg/kg i.p.). Catheters (each consisting of 5 cm polyethylene-10 tubing connected to 15 cm polyethylene-50 tubing) were placed in the abdominal aorta and vena cava via the femoral artery and vein for measurement of BP and i.v. administration of drugs, respectively. The arterial catheter was connected to a BP transducer (model P23XL; Astro-Med, West Warwick, RI, USA) that was attached through MLAC11 Grass adapter cable to a computerized data acquisition system with LabChart-7 pro software (Power Lab 4/35, model ML866/P; AD Instruments Pty Ltd., Castle Hill, Australia). The LabChart-7 Pro software computes the HR by applying the cyclic measurement function. Finally, catheters were tunneled subcutaneously, exteriorized at the back of the neck between the scapulae, flushed with heparin (0.2 ml; 100 U/ml), and plugged by stainless steel pins. Each rat received an i.m. injection of 60,000 U of benzathine benzyl penicillin. Experiments started 2 days later in conscious rats.

2.3. Time-domain analysis of HRV

Two time-domain measures of the cardiac autonomic activity were employed, the standard deviation of beat-to-beat intervals (SDNN) and the root mean square of successive beat-to-beat differences in R-R interval durations (rMSSD) (Omar and El-Mas, 2004; Stein et al., 1994). The RR intervals were computed from the heart rate (i.e. the reciprocal of heart rate in ms). The SDNN is comparable to the total power of the spectrum of RR variability, which measures the overall autonomic balance of the heart. The rMSSD is largely validated as a measure of the parasympathetic input to the heart and, therefore, correlates with the high frequency power of the spectrum (Stein et al., 1994; Sgoifo et al., 1997). SDNN and rMSSD were measured before (baseline) and at 10 min intervals after drug treatments. For each time point, the 5-min values of each variable were averaged.

2.4. Frequency-domain analysis of HRV

Spectral hemodynamic fluctuations, quantitative indices of cardiac autonomic control (Stein et al., 1994; El-Mas and Abdel-Rahman, 2007), were used to reflect changes in sympathetic and vagal outflows. Hemodynamic variability was analyzed in the frequency domain using FFT algorithms of R-R data series (Stein et al., 1994; El-Mas and Abdel-Rahman, 2007). The FFT algorithm for direct transformation of data points into power spectral density graphs was used. Data were interpolated to obtain equally spaced samples with an effective sampling frequency of 10 Hz (0.1 s). A second-order interpolation was employed to fit a smooth curve to the existing data points and produce a smoother visual representation of data. The evenly-spaced (equidistant) sampling allowed direct spectral analysis using FFT algorithm. Spectra were integrated into 2 specific frequency bands, LF (0.25–0.75 Hz) and HF (0.75–3 Hz) bands. Spectral data were estimated before (baseline) and at 10 min intervals after drug treatments. For each time point, the 5-min values of each variable were averaged.

2.5. Protocols and experimental groups

2.5.1. Effect of CSA on hemodynamic and autonomic responses to endotoxemia

Two sets of experiments were used to investigate the effect of CSA on the cardiovascular and autonomic effects of endotoxemia. In the first, 5 groups of rats (n=6–8 each) were employed to investigate the effect of subsequent CSA administration on LPS responses. Rat groups were allocated to receive one the following i.v. regimens: (i) vehicle group (saline + cremophor), (ii) saline + CSA (10 mg/kg) (Zhang et al., 2000), (iii) LPS (10 mg/kg) (Mori et al., 2010) + saline, (iv) LPS + cremophor, or (v) LPS + CSA. A period of 30 min was allowed between the two treatments of each regimen. CSA or its vehicle cremophor was infused at a rate 0.1 ml/min followed by a flush volume of 0.2 ml saline. Hemodynamic monitoring continued for 60 min after the second treatment (CSA or cremophor). Changes in MAP, HR, and time- and frequency-domain indices of cardiovascular autonomic control caused by the second drug treatments (CsA or cremophor) compared with pretreatment levels were computed at 10 min intervals. A blood sample (0.8 ml) was withdrawn from the arterial line of each rat 60 min post i.v. LPS (10 mg/kg) for determination of serum TNF-α and IL-6. Blood was allowed to coagulate at room temperature for 10–20 min and then centrifuged at 5000 rpm for 10 min. The supernatant (serum) was aspirated and stored at −80 °C until used for ELISA determination of TNF-α and IL-6 (Glory science Co., Ltd, Hangzhou, China) as instructed by the manufacturer.

In the second set of experiments, another 3 groups of rats (n=6 each) were used to assess whether the effects of CSA could be replicated when it was administered prior to LPS. In this group, CSA (10 mg/kg) or its vehicle cremophor was intravenously injected 15 min before LPS and hemodynamic monitoring continued for 90 min thereafter.

2.5.2. Role of central PI3K/MAPKs/sGC signaling in the LPS-CSA interaction

This experiment investigated the roles of PI3K and downstream MAPKs ($MAPK_{JNK}$, $MAPK_{ERK}$, and $MAPK_{P38}$)/sGC signaling in central neurons to the effect of systemically administered CSA on the hemodynamic and autonomic effects of LPS. Ten groups of rats (n =6–8 each) were allocated to receive one of the following drug