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Effect of endotoxin on heart rate dynamics in rats with cirrhosis

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ABSTRACT

Reduced heart rate variability (HRV) is a hallmark of systemic inflammation which carries negative prognostic information in sepsis. Decreased HRV is associated with partial uncoupling of cardiac pacemaker from cholinergic neural control during systemic inflammation. Sepsis is a common complication in liver cirrhosis with high mortality. The present study was aimed to explore the hypothesis that endotoxin uncouples cardiac pacemaker from autonomic neural control and reduces HRV in an experimental model of cirrhosis. Cirrhosis was induced by surgical ligation of the bile duct in rats. Cirrhotic rats were given intraperitoneal injection of either saline or lipopolysaccharide (endotoxin, 1 mg/kg). Changes in HRV indices were studied in conscious rats using implanted telemetric probes. The atria were isolated and chronotropic responsiveness to cholinergic stimulation was assessed in vitro. Endotoxin injection induced a significant tachycardia and decreased short-term and long-term HRV indices in control rats. However, endotoxin was unable to increase heart rate in cirrhotic animals. In contrast with control rats, endotoxin induced biphasic changes in short-term HRV in cirrhotic rats. Acute endotoxin challenge reduced long-term HRV with 60-min delay in comparison with control animals. Endotoxin injection was associated with a significant hypo-responsiveness to cholinergic stimulation in control rats in vitro. Endotoxin did not change atrial chronotropic responsiveness to cholinergic stimulation in cirrhotic rats. Our data shows that cirrhosis is associated with development of tolerance to cardiac chronotropic effect of endotoxin in rats.

1. Introduction

Sepsis is a common complication of liver cirrhosis and is linked to high mortality in this patient population (Wong et al., 2005; O'Brien et al., 2012). Experimental studies have shown that there is a marked sensitivity to bacterial endotoxin in cirrhotic rats which is associated with enhanced production of pro-inflammatory cytokines as well as increased lipid peroxidation and protein S-nitrosation (Harry et al., 1999; Ottesen et al., 2001). Besides, sepsis and cirrhosis exhibit common features which may be in part related to endotoxemia and translocation of bacterial products in cirrhosis (Thalheimer and Burroughs, 2008).

Cardiac rhythm exhibits a complex dynamics in physiological state as the indicator of a complex interaction between cardiac pacemaker cells and the autonomic nervous system (Goldberger et al., 2002). Sepsis and cirrhosis are both characterized by alteration in heart rate dynamics and reduced heart rate variability (HRV) (Goldstein et al., 1998; Mani et al., 2009). Moreover, reduced HRV carries negative prognostic value in these clinical conditions (Goldstein et al., 1998; Mani et al., 2009). Recent studies have shown that HRV is reduced in clinical conditions which are associated with systemic inflammation (Aronson et al., 2001; González-Clemente et al., 2007; Tateishi et al., 2007) and circulating levels of interleukin-6 (IL-6) correlate significantly with indices of depressed HRV in various clinical conditions such as sepsis, liver cirrhosis and diabetes (Aronson et al., 2001; González-Clemente et al., 2007; Tateishi et al., 2007; Mani et al., 2009; Hajiasgharzadeh et al., 2011). The underlying mechanism of increased cardiac cycle regularity during systemic inflammation is not well understood. According to some scientists namely Pincus, greater regularity in a complex system could indicate uncoupling of the system's components (Pincus, 1994; Buchman, 2002). Also, Godin et al. (1996) observed that injection of bacterial endotoxin causes mild uncoupling of autonomic regulation manifested as the loss of cardiac cycle variability in humans. Furthermore, we recently reported that systemic inflammation is linked to reduced cardiac responsiveness to cholinergic stimulation that may lead to partial uncoupling of cardiac pacemaker cells from autonomic parasympathetic neural control (Gholami et al., 2012). Another recent study showed that IL-6 receptor is expressed in mouse atria and incubation of isolated atria with recombinant IL-6 entails impaired negative chronotropic responsiveness to cholinergic stimulation (Hajiasgharzadeh et al., 2011). These reports indicate that uncoupling of cardio-regulatory mechanisms may occur

Abbreviations: BDL, bile duct ligated; DFA, detrended fluctuation analysis; HRV, heart rate variability; IL-6, interleukin 6; LPS, lipopolysaccharide; SHAM, sham-operated; SOCS1, suppressor of cytokine signaling-1; TGF-β, transforming growth factor-beta.

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at pacemaker level and can explain decreased HRV during systemic inflammation (Hajiasgharzadeh et al., 2011; Gholami et al., 2012).

Moorman and colleagues have extensively studied the indices that describe heart rate dynamics in neonates and reported that HRV analysis can be used as an aid for early diagnosis of sepsis (Lake et al., 2002). What is more, we have observed that the relative risk of death increased by 7.7% for every millisecond drop in an HRV variable in patients with cirrhosis (Mani et al., 2009). Although it is well established that HRV is reduced in cirrhosis, heart rate dynamics in response to endotoxin challenge has not been investigated in cirrhotic subjects. The present study was aimed to explore the hypothesis that whether or not the inflammatory response induced by administration of endotoxin uncouples cardiac pacemaker from autonomic neural control and exacerbates HRV response in an experimental model of cirrhosis.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (body weight 230–250 g) were obtained from Razi Institute (Hesarak, Iran). All animal maintenance procedures were in accordance with recommendations established by the Animal Ethics Committee of Tarbiat Modares University, Home Office (UK) as well as the United States NIH guidelines (publication no. 85-23). Cirrhosis was induced by ligation of bile duct as described in literature (Ebrahimkhani et al., 2006). In brief, animals were anesthetized using ketamine (100 mg/kg) and xylazine (10 mg/kg) and underwent bile duct ligation surgery. Bile duct was doubly ligated 3–5 mm above its intrapancreatic portion and transected between the two ligatures. All studies were performed 28 days after bile duct ligation. Sham operated rats were used as control. Sample sizes were calculated to achieve 90% power. In total, we used 86 rats to complete the experiments (50 rats for in vivo HRV study, 24 rats for in vitro study and 12 rats for real time RT-PCR assay).

2.2. Telemetric recording of electrocardiogram from conscious rats

A dorsally mounted radio frequency transmitter and wire leads (lead I configuration) were implanted subcutaneously under anesthesia using ketamine and xylazine. Bile duct ligation or sham surgeries were carried out at the time of implantation of the telemetric probe. 28 days after operation, electrocardiogram (ECG) and body temperature were recorded using a telemetry system (Data Sciences International, St. Paul, Minnesota, USA) connected to a Powerlab data acquisition system (ADInstruments, Sydney, Australia). Animals received intraperitoneal injection of either saline or endotoxin (LPS; *Salmonella typhimurium* lipopolysaccharide, 1 mg/kg dissolved in isotonic saline) (Gholami et al., 2012). All recordings were started at 8 AM and were continued for 5 h.10 rats were used in each of the cirrhotic (saline), cirrhotic (LPS) and control (LPS) groups. 20 rats were used in control (saline) group.

2.3. Data acquisition

The ECG data were exported at a sampling rate of 10 kHz using a Powerlab data acquisition system. The R peaks were detected and the R–R interval series were generated using an ad hoc computer program. The R–R interval series was visually inspected and 1750 artifact-free continuous R–R intervals were selected for HRV analysis.

2.4. HRV analysis

The standard deviation of the R–R intervals (SDNN) was calculated on the selected artifact-free trace and used as a measure of total HRV. Nonlinear measures of HRV provide information on the structure or complexity of the R–R time-series. In the present study nonlinear measures of HRV were assessed using Poincaré plot as well as detrended fluctuation analysis (DFA).

2.4.1. Poincaré plot

The Poincaré plot is a graphical representation of the correlation between consecutive R–R intervals [x-axis: R–R (n): y-axis: R–R (n + 1)]. The standard deviation of the points perpendicular to the line of identity (SD1) describes short-term variability which is mainly related to the effects of respiration on vagal drive (Tulppo et al., 1996); the standard deviation along with the line of identity (SD2) describes the long-term R–R interval variations and accounts for all other heart rate changes. The parameters SD1 and SD2 were calculated applying software developed by Niskanen et al. (2004).

2.4.2. Detrended fluctuation analysis (DFA)

DFA quantifies fractal-like correlation properties on the time-series (Peng et al., 1995). In this method, the root mean square of fluctuation of the integrated and detrended data is measured within observation windows of various sizes and then plotted against window size on a log–log scale. A linear relationship between log (fluctuation) and log (window size) indicates the presence of scaling which serves as a characteristic of a fractal-like time-series (Peng et al., 1995). The scaling exponent alpha (α) indicates the slope of this line. An $\alpha = 0.5$ indicates white noise (uncorrelated random data). An α greater than 0.5 and less than or equal to 1.0 indicates persistent long-range power-law correlations in which a large (compared to the average) interbeat interval is more likely to be followed by a large interval. A special case $\alpha = 1$ corresponds to $1/f$ noise. $\alpha = 1.5$ also corresponds to brown noise (integration of white noise).

In order to estimate sympathovagal balance spectral analysis of HRV was also calculated using FFT (fast Fourier transform) algorithm. Power spectrum analysis of HRV was calculated in two spectral components using software developed by Niskanen et al. (2004) as follows: 1. High frequency component (HF: 1–3 Hz in rats) which is caused by an inhibition of the vagal tone during inspiration. 2. Low frequency component (LF; 0.04–1 Hz in rats) which is mostly representative of cardiac sympathetic activity in rats. The LF/HF ratio was also calculated and used as a measure of cardiac sympathovagal balance (Mani et al., 2006).

2.5. In vitro study

28 days after bile duct ligation or sham surgery, animals were divided into two groups that were intraperitoneally injected with LPS (*S. typhimurium* lipopolysaccharide 1 mg/kg) or saline (Gholami et al., 2012). 2 h after injection, spontaneously beating atria were isolated in order to study chronotropic responsiveness to cholinergic stimulation ($n = 6$ in each group). In brief, the atria were isolated in cold oxygenated physiological solution and suspended under isometric tension of 10 mN in a 25-ml organ bath glass chamber as described (Gholami et al., 2012). The temperature of bathing solution was 37.0 ± 0.1 °C and pH was 7.4. The composition of physiological solution (in mM) was as follows: NaCl 112, KCl 5, $CaCl_2$ 1.8, $MgCl_2$ 1, NaH_2PO_4 0.5, KH_2PO_4 0.5, $NaHCO_3$ 25, Glucose 10 and EDTA 0.004. The solution was oxygenated with a gas mixture of 95% O_2 and 5% CO_2. The signals were digitized at a sampling rate of 10 kHz and displayed on a Powerlab system. The atrium, which contains the sinoatrial node, was used for recording of the spontaneous atrial beating. Spontaneous contractions were recorded with an isometric transducer. To avoid artifact evoked by dissection, an equilibration period of 30 min was allowed before evaluation of the spontaneous contractions. The responsiveness of isolated atria to cholinergic stimulation was evaluated by addition of cumulative concentrations of carbacholine (10^{-9} to 10^{-5} M) (Gholami et al., 2012). IC_{50} values (the concentration of where the beating rate is reduced by half) were calculated using nonlinear regression analysis.