Autonomic Neuroscience: Basic and Clinical 189 (2015) 83–86

Contents lists available at ScienceDirect

Autonomic Neuroscience: Basic and Clinical

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

Short communication

The effect of endotoxin on heart rate dynamics in diabetic rats

Morvarid Meamar [a], Tara Dehpour [b], Roham Mazloom [a], Fatemeh Sharifi [a], Mohammad R. Raoufy [a], Ahmad R. Dehpour [b], Ali R. Mani [a,b,*,1]

[a] Department of Physiology, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[b] Experimental Medicine Research Center, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article history:
Received 21 September 2014
Received in revised form 10 December 2014
Accepted 28 December 2014

Keywords:
Endotoxin
Diabetes
Heart rate variability

ABSTRACT

The effect of endotoxin on heart rate variability (HRV) was assessed in diabetic and controls rats using a telemetric system. Endotoxin induced a reduction in sample entropy of cardiac rhythm in control animals. However, this effect was significantly blunted in streptozotocin-induced diabetic rats. Since uncoupling of cardiac pacemaker from cholinergic control is linked to reduced HRV in endotoxemia, chronotropic responsiveness to cholinergic stimulation was assessed in isolated atria. Endotoxemia was associated with impaired responsiveness to carbacholine in control rats. However, endotoxemia did not impair cholinergic responsiveness in diabetic atria. These findings corroborates with development of endotoxin tolerance in diabetic rats.

1. Introduction

Heart rate variability (HRV) analysis has extensively been used in a variety of clinical settings such as diagnosis of autonomic neuropathy, fetal heart rate monitoring as well as diagnosis of sepsis (Altimiras, 1999; Lake et al., 2002). Sepsis is associated with reduced HRV and a significant regularization of cardiac cycle variability (Lake et al., 2002; Tateishi et al., 2007). Moreover, HRV monitoring is shown to have diagnostic and prognostic values in patients with sepsis (Ahmad et al., 2009; Goldstein et al., 1998). For example HRV monitoring has the potential to be used for early diagnosis of sepsis in neonates as well as patients with immune suppression (e.g. patients with bone marrow transplantation) (Ahmad et al., 2009; Griffin et al., 2005). In these medical conditions clinical manifestations of systemic inflammation appear in late stages of sepsis and early diagnosis may reduce mortality (Griffin et al., 2005). The advantage of using HRV monitoring is that it is a non-invasive method that gives information on complexity of cardiovascular regularity mechanisms (Shirazi et al., 2013).

It appears that uncoupling of cardiac pacemaker from cholinergic neural control plays a role in reduced heart rate variability in experimental models of sepsis (Eftekhari et al., 2013). Recent studies provide evidence for an alteration in cardiac pacemaker dynamics during systemic inflammation (Mazloom et al., 2014; Zorn-Pauly et al., 2007). Mazloom et al. (2014) reported that acute endotoxin challenge reduces the controllability of isolated perfused hearts. Injection of bacterial lipopolysaccharide (endotoxin) in rats could also impair cardiac chronotropic responsiveness to cholinergic activation (Gholami et al., 2012). Moreover, incubation of mouse isolated atria with interleukin-6 significantly reduces the effect of cholinergic agonist on cardiac pacemaker rate (Hajiasgharzadeh et al., 2011). These reports suggest that cardiac pacemaker is a target during systemic inflammation and altered pacemaker dynamics may explain reduced HRV in experimental models of sepsis.

Many chronic medical conditions (e.g. diabetes, congestive heart failure and liver cirrhosis) are associated with increased plasma cytokine level and reduced HRV (Aronson et al., 2001; González-Clemente et al., 2007; Mani et al., 2009). However it is not known how the HRV of patients with these underlying medical conditions would respond to sepsis (or endotoxin challenge). This is particularly important in patients with diabetes who might be susceptible to develop sepsis due to diabetic complications (e.g. diabetic foot). If HRV monitoring is going to be used as a tool for diagnosis of sepsis, it is crucial to understand the limitation of this method in patients with underlying medical conditions such as diabetes. In a preliminary study to investigate the effect of acute endotoxin challenge on HRV in diabetic rats, we observed that diabetic rats exhibit tolerance to the chronotropic effect of endotoxin. This short communication reports our data on the effect of endotoxin on heart rate dynamics in diabetic rats.

2. Materials and methods

2.1. Telemetric recording of electrocardiogram from conscious rats

Male Sprague–Dawley rats (body weight of 230–250 g) were obtained from the Razi Institute (Hesarak, Iran). All animal procedures were in

Abbreviations: HRV, heart rate variability; LPS, lipopolysaccharide; STZ, streptozotocin

* Corresponding author at: Experimental Medicine Research Center, Tehran University of Medical Sciences, Tehran, Iran.
E-mail address: a.r.mani@ucl.ac.uk (A.R. Mani).
[1] Present address: Division of Medicine, University College London, London, UK.

http://dx.doi.org/10.1016/j.autneu.2014.12.006
1566-0702/

accordance with recommendations by the ethics committee of Tarbiat Modares University. A dorsally mounted radio frequency transmitter (Data Sciences International, St. Paul, Minnesota, USA) was implanted subcutaneously under anesthesia using ketamine and xylazine (Mazloom et al., 2013). Two weeks after the operation, diabetes was induced by a single intraperitoneal injection of streptozocin (STZ, 60 mg/kg, dissolved in 0.1 M citrate buffer, pH 4.5). To confirm the induction of diabetes, fasting blood glucose concentration were measured 3 and 14 days after STZ injection using a glucometer. Vehicle (citrate buffer pH: 4.5) treated rats were used as control. Fourteen days after induction of diabetes, electrocardiogram (ECG) was recorded using a telemetry system connected to a PowerLab data acquisition system (ADInstruments, Sydney, Australia). Animals received intraperitoneal injection of either saline or lipopolysaccharide (LPS; *Salmonella typhimurium* endotoxin, 1 mg/kg dissolved in isotonic saline) (Gholami et al., 2012). All recordings were started at 8 AM and were continued for 24 h. Six to eight rats were used in each group.

2.2. Data acquisition and HRV analysis

The ECG data were exported at a sampling rate of 10 kHz using a Powerlab data acquisition system. The R–R interval series were generated using Chart software (version 5, ADInstruments). The R–R interval series was visually inspected and 1800 artifact-free continuous R–R intervals were selected for calculation of sample entropy as described (Gholami et al., 2012; Lake et al., 2002).

2.3. In vitro study

A separate group animals were used for in vitro study. 14 days after STZ or vehicle administration, animals were divided into two groups that were intraperitoneally injected with LPS (1 mg/kg) or saline. Three hours after injection, spontaneously beating atria were isolated 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 (Mani 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 power lab system. Spontaneous contractions were recorded with an isometric transducer. 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) to the organ bath (Mani et al., 2012).

2.4. Statistical analysis

The results are presented as means ± standard deviation (SD). Sample size was calculated to achieve 90% power. Two-way ANOVA was used to assess the effect of diabetes or time after LPS injection on sample entropy. Bonferroni post-test was then applied for multiple comparisons. Student's *t*-test was used to compare fasting blood glucose and basal heart rate between diabetic and control rats. P-values less than 0.05 were considered statistically significant.

3. Results and discussion

Manifestations of diabetes appeared couple of days after STZ injection and rats with fasting blood glucose more than 250 mg/dl were enrolled in the study. Two weeks after STZ injection, fasting blood glucose was significantly higher in the STZ-treated animals in comparison with vehicle-treated rats (559 ± 22 versus 121 ± 20 mg/dl, $P < 0.001$). The basal heart rate of conscious animals was assessed using a telemetric system. The basal heart rate of the diabetic rats was 225 ± 18 beat/min which was significantly lower than the healthy rats (331 ± 17 beat/min, $P < 0.001$). This data corroborates with previous reports that indicate bradycardia in STZ-treated rats (Malone et al., 2007). It appears that type I diabetes in rats is associated with slow heart rate due to slow pacemaker activity (Howarth et al., 2007). Our results on isolated atria also showed that the basal atrial beating rate was slower than normal condition (266 ± 15 versus 299 ± 22 in diabetic and control respectively, $P < 0.001$). The main purpose of our study was to investigate the effect of acute endotoxin challenge on heart rate dynamics in diabetic rats. Fig. 1 represents the effect of endotoxin (LPS, 1 mg/kg) on sample entropy of the inter-beat intervals starting from one hour before endotoxin injection up to 24 h post-LPS injection. Acute endotoxin challenge in control rats was associated with a significant reduction in sample entropy ($F = 8.20$, $P < 0.01$). As shown in Fig. 1, LPS was unable to induce a prominent reduction in sample entropy in diabetic rats ($F = 3.52$, $P > 0.05$). There was a significant difference in sample entropy between non-diabetic and diabetic rats in response to LPS (Two-way ANOVA, $F_{\text{control versus diabetic}} = 22.57$, $P < 0.001$). We also tested the effect of saline injection on sample entropy in both control and diabetic rats. Saline injection did not have a significant effect on sample entropy in neither the diabetic nor control group (data not shown). We also looked at other HRV parameters such as SD_1 (short-term HRV) and SD_2 (long-term HRV) using Poincaré plot. Our data showed that while endotoxin reduced SD_1 and SD_2 in the control group, this effect was blunted in the diabetic rats (data not shown).

We used sample entropy as a non-linear HRV index that has already been used for diagnosis of sepsis in humans (Lake et al., 2002). Sample entropy measures the negative logarithmic likelihood of the repetition of patterns in a time series (e.g. heart rate). In other words, sample entropy calculates the probability that epochs of window length m that are similar within a tolerance r remain similar at the next point (Richman and Moorman, 2000). The advantages of using sample entropy in our study are the following: a. Sample entropy deals with the pattern of cardiac rhythm. Therefore, unlike common linear HRV indices (such as the standard deviation of R–R intervals) it does not depend on basal heart rate. Since our experimental model was associated with bradycardia, sample entropy was a better index than conventional HRV indices. b. Sample entropy has the same physical dimension as

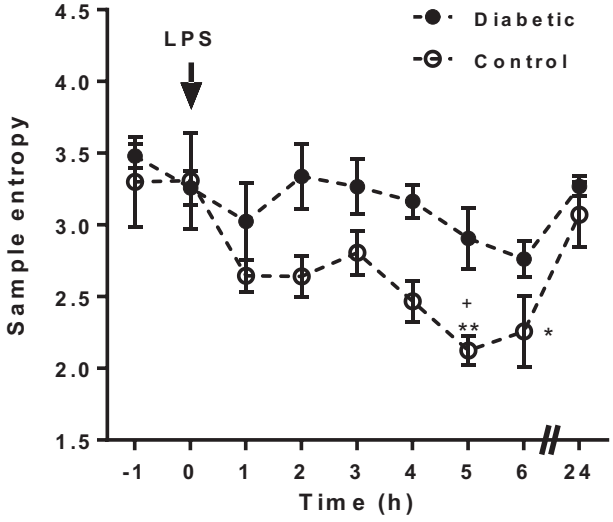

Fig. 1. Time-dependent effect of endotoxin (LPS) on sample entropy in control or diabetic rats. Arrow indicates the time of LPS injection. Data are expressed as Mean ± standard error of mean. * $P < 0.05$, ** $P < 0.01$ in comparison with time 0 (control group), + $P < 0.05$ compared with diabetic group (time 5 h).