Autonomic Neuroscience: Basic and Clinical 180 (2014) 17–23

Contents lists available at ScienceDirect

Autonomic Neuroscience: Basic and Clinical

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

Right atrium cholinergic deficit in septic rats

Paola Contreras *, Eduardo R. Migliaro, Bruno Suhr

Departamento de Fisiología, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay

ARTICLE INFO

Article history:
Received 24 May 2013
Received in revised form 3 October 2013
Accepted 6 October 2013

Keywords:
Sepsis
Autonomic dysfunction
Heart rate variability
Telemetry

ABSTRACT

Heart rate variability (HRV) is mainly determined by the influence of both branches of the Autonomic Nervous System over the sinus node. Low HRV has been associated with a worse prognosis in patients with sepsis. The objective of this study was to explain the reduction in HRV during experimental sepsis in adult rats. We recorded the heart's electrical activity by telemetry in conscious unrestrained male rats before and 1 day after the induction of peritonitis ($N = 39$) or sham peritonitis ($N = 15$). Then, we analyzed the chronotropic responsiveness of the isolated heart to the autonomic neurotransmitters and determined catecholamine concentrations in blood plasma and acetylcholine and choline concentrations in the right atrium. The surviving septic rats ($N = 33$) had increased heart rate (HR) and diminished HRV. Despite the higher HR in situ, the spontaneous basal HR in septic and sham isolated hearts was the same. The isolated septic hearts showed acetylcholine hypersensitivity ($\log(IC_{50},M) = -7.2 \pm 0.2$ vs. -6.0 ± 0.4, $P = 0.025$) and lower concentrations of choline in their right atriums (in nMol/mg protein: 0.6 ± 0.1 vs. 1.6 ± 0.6, $P = 0.013$). Norepinephrine concentration in blood plasma from septic rats was higher (in ng/ml: 29.2 ± 8.4 vs. 5.8 ± 4.1, $P = 0.019$). In conclusion, septic rats present a deregulation of the autonomic nervous system, not only sympathetic overexcitation but also parasympathetic dysfunction.

1. Introduction

Sepsis is a clinical syndrome defined by the presence of both infection and systemic inflammatory response (Levy et al., 2003). We have previously shown that in an otherwise similar group of septic patients, reduced heart rate variability (HRV) was associated with a worse prognosis, leading to multiple organ dysfunction syndrome and high mortality (Pontet et al., 2003).

HRV reflects the continuous oscillation of the RR intervals around its mean value, and it is mostly determined by the modulation of the sinus node activity by both branches of the autonomic nervous system. A lower HRV has been described in clinical and experimental sepsis (Pancoto et al., 2008). The reduction in HRV during sepsis has been ascribed to the uncoupling of the autonomic and cardiovascular systems (Godin et al., 1996). This could be due to refractoriness of the heart to the autonomic neurotransmitters (Hajiasgharzadeh et al., 2011; Haddadian et al., 2013) and/or to an altered activity of the autonomic nervous system (Werdan et al., 2009).

Partial uncoupling of isolated atria from endotoxemic rats to cholinergic stimulation has been described recently using non-lethal doses of lipopolysaccharide (LPS) (Gholami et al., 2012).

The enhanced sympathetic activity in sepsis has been time correlated with tachycardia in LPS treated rats (Vayssettes-Courchay et al., 2005). In accordance with this, a huge increase in plasma norepinephrine levels at 24 h after cecal ligation and puncture (CLP) in rats has been reported (Berger et al., 2011; Kovarik et al., 1987).

The activation of vagal afferent and efferent signaling has been related to the inflammatory reflex that attenuates the host response to pathogens that might itself become deleterious if left uncontrolled (Tracey, 2007). Lack of this activation or even depression of the cholinergic activity (e.g., by desensitization (Fairchild et al., 2011)) might contribute to decreased HRV and worse prognosis in severe sepsis.

Our main objective was to explain the reduction in HRV during sepsis. We first measured HRV in conscious rats, before and 1 day after the induction of polymicrobial sepsis by peritonitis. Then, we analyzed the chronotropic responsiveness of their isolated hearts to autonomic neurotransmitters or indirectly assessed their autonomic nervous system efferent activity by determining the catecholamine concentrations in their plasma and the acetylcholine and choline concentrations in their right atria.

2. Materials and methods

2.1. Animals

This study was approved by the Comité de Ética en el Uso de Animales (CEUA, Uruguay), protocol number # A5373-01. Animal care

* Corresponding author at: Departamento de Fisiología, Facultad de Medicina, Universidad de la República. Avenida General Flores 2125. Montevideo, 11800, Uruguay.
E-mail addresses: contreras@fmed.edu.uy, contrechai@gmail.com (P. Contreras).

1566-0702/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.autneu.2013.10.002

and handling were in accordance with the National Institutes of Health Guidelines.

Fifty-four adult Wistar derived albino male rats (13.5 ± 0.3 weeks old and weighing 312 ± 4 g) were provided by the *vivarium* located in the same building where the laboratory is situated. Each week, two rats were brought to the lab and housed in individual cages at controlled room temperature and natural photoperiod conditions. The two rats were randomly assigned to the sepsis ($N = 39$) or sham protocols ($N = 15$). Rodent chow and water were available ad libitum during the entire experimental period (4 days).

2.2. In vivo study: heart rate and HRV evaluation in conscious unrestrained rats

We recorded the heart electrical activity (1-lead ECG) of all the rats included in this study ($N = 54$). A dorsally mounted radiofrequency transmitter (CA-F40, Data Sciences International DSI, St. Paul, MN) with two wire leads was implanted subcutaneously in the rats under anesthesia with isoflurane (2.5% at 0.5 lpm O_2). The electrodes were sutured to the chest muscles (lead II-like configuration) and the skin was closed with metal clips. The rats were allowed to recover for 48 h.

The ECG of the conscious, freely moving rats was recorded for 60 min in the morning (8–11 am) before and 1 day after the induction of sepsis.

We used a 1208 FS A/D converter card (Measurement Computing, Norton, MA) and homemade software. The sampling frequency was 1000 Hz. Automatic detection of R waves and measurement of RR intervals were performed offline with Spike2 software (version 6.07, Cambridge Electronic Design, Cambridge, UK). The detection was visually inspected and corrected if necessary. Another software was used for the filtering of the RR time series (Machado et al., 2000) and for the calculation of the mean heart rate (HR) and statistical HRV indices. The absence of outliers after appropriate filtering was confirmed by graphical representation of all RR intervals considered for HRV analysis. Each RR interval was plotted as a function of time (tachogram) or as a function of the preceding RR (Poincaré plot). We calculated SDNN (the standard deviation of all normal RR intervals) and RMSSD (square root of the mean squared successive differences of RR intervals) (Aubert et al., 1999). SDNN is an index of global variability, while RMSSD evaluates short-term variability on a beat-to-beat basis (parasympathetic influences (Hill and Siebenbrock, 2009)). As SDNN is affected by HR, the coefficient of variation (CV) was also calculated as SDNN (ms) / RR interval (ms) × 100.

Although frequency-domain analysis is generally used for autonomic nervous system evaluation, we did not use it for the following reasons: the procedure for the estimation of these indices is not standardized for rodents, non-stationary time-series yield non-reliable results and besides, it has been argued that this method does not provide additional information beyond that obtained by statistical indices in rodent sepsis (Fairchild et al., 2011; Stauss, 2003). Furthermore, Stauss has reported that these indices do not always reflect autonomic nervous system activity, and that simple statistics of HRV reliably predict the prognosis of various diseases (Stauss, 2003).

2.3. Sepsis induction

Intra-abdominal sepsis was induced by cecal ligation and fecal inoculum (CLF, $N = 39$). Under anesthesia with isoflurane, an abdominal incision was made and the cecum was exposed. Double ligation of the cecum was performed to provide a source of necrotic tissue that is often found in clinical sepsis (Hubbard et al., 2005). The bowel was returned without perforation. Instead, fecal slurry (300 mg of autologous feces in 5 ml of sterile saline solution) was spilled in the peritoneum (300 mg/kg) (Chopra and Sharma, 2007; Chopra et al., 2011). The abdominal cavity was closed in two layers.

Sham animals ($N = 15$) were submitted to laparotomy and the cecum was manipulated, but not tied. An equivalent volume of normal saline was spilled in the peritoneum instead.

At the end of the surgery, all rats received a subcutaneous injection of normal saline (Hubbard et al., 2005) to reach a dose of volume replacement therapy of 10 ml/kg.

2.4. In vitro study: chronotropic responsiveness of isolated hearts to Autonomic Nervous System neurotransmitters

2.4.1. Chronotropic responsiveness of isolated hearts to norepinephrine

We evaluated the effect of sepsis on the chronotropic responsiveness of isolated hearts to norepinephrine stimulation 1 day after the induction of sepsis and sham sepsis ($N = 10$ and 5 respectively) once the in vivo ECG was recorded.

Under anesthesia with isoflurane, the thorax was opened and the heart was exposed. The heart was excised and mounted in a Langendorff setup for perfusion through the aorta (constant flow of 9 ml/min) with Tyrode solution (composition in mmol/l: NaCl, 140; KCl, 5.4; $MgCl_2$, 1; $CaCl_2$, 2; NaH_2PO_4, 0.33; glucose, 10; and HEPES, 10; pH adjusted to 7.4 with NaOH at 37 °C). The solution was gassed with 100% O_2.

Two wire electrodes were attached to the right atrium and pulmonary cone, and the spontaneous electrical activity of the heart was recorded using a Tecnomed electrical amplifier, LabMaster A/D converter, and Axotape software (the sampling frequency was 500 Hz). Recordings were analyzed offline with Spike2 software. Automatic detection of the first wave of the electrical recording was performed to consider the sinus rhythm.

Dose–response results were obtained by sequential perfusion with five norepinephrine solutions at increasing concentrations for 5 min each (10^{-8}–10^{-4} M). Adrenergic responsiveness was examined in the presence of 10^{-6} M atropine (15 min before the first dose and during the doses). The cholinergic antagonist was added to prevent effects of possible local neurotransmitter release (Hicks et al., 1997), and it had no effect on basal pacemaker rate. The maximum HR reached with each dose of norepinephrine was considered for analysis. Basal HR (spontaneous beating rate) was considered as the average HR for the last 5 min of the 25-min stabilization period (before changing to the antagonist). Control HR for dose–response analysis was calculated as the average HR for the 5-min period prior to perfusion with the lowest dose of neurotransmitter (after 10 min with atropine).

2.4.2. Chronotropic responsiveness of isolated hearts to acetylcholine

We evaluated the effect of sepsis on the chronotropic responsiveness of isolated hearts to acetylcholine stimulation 1 day after the induction of sepsis and sham sepsis ($N = 10$ and 5 respectively) once the in vivo ECG was recorded.

The protocol was the same as described above for norepinephrine, but the dose–response results were obtained by sequential perfusion with three increasing doses of acetylcholine (10^{-8}–10^{-6} M) for 5 min each. Cholinergic responsiveness was examined in the presence of an adrenergic antagonist (10^{-6} M nadolol). The minimum HR achieved with each dose was considered for analysis.

2.5. Quantification of autonomic nervous system neurotransmitters

Fifteen rats were included in this protocol, 1 day after the induction of sepsis and sham sepsis ($N = 10$ and 5, respectively) once the in vivo ECG was recorded.

The rats were anesthetized with isoflurane and their hearts were exposed. Blood samples for epinephrine and norepinephrine quantification were collected from the right ventricles. The rats were euthanized when the right atria were isolated from the hearts for