Digitoxin improves cardiovascular autonomic control in rats with heart failure

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Digitoxin improves cardiovascular autonomic control in rats with heart failure

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Running Head: Digitoxin improves cardiovascular autonomic control

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ABSTRACT

The effects of chronic treatment with digitoxin on arterial baroreceptor sensitivity for heart rate (HR) and renal sympathetic nerve activity (rSNA) control, cardiopulmonary reflex, and autonomic HR control in an animal model of heart failure were evaluated. Wistar rats were treated with digitoxin, which was administered in their daily feed (1mg/Kg/day), for 60 days. The following three experimental groups were evaluated: Sham, heart failure (HF), and HF treated with digitoxin (HF+DIG). We observed an increase in rSNA in the HF group (190±29pps, n=5) compared with the Sham group (98±14pps, n=5). Digitoxin treatment prevented an increase in rSNA (98±14pps, n=7). Therefore, arterial baroreceptor sensitivity was decreased in the HF group (1.24±0.07bpm/mmHg, n=8) compared with the Sham group (2.27±0.23bpm/mmHg, n=6). Digitoxin did not alter arterial baroreceptor sensitivity in the HF+DIG group. Finally, the HF group showed an increased low frequency band (LFb: 23±5ms², n=8) and a decreased high frequency band (HFb: 77±5ms², n=8) compared with the Sham group (LFb: 14±3ms²; HFb: 86±3ms², n=9); the HF+DIG group exhibited normalized parameters (LFb: 15±3ms²; HFb: 85±3ms², n=9). In conclusion, the benefits of decreasing rSNA are not directly related to improvements in peripheral cardiovascular reflexes; such occurrences are due in part to changes in the central nuclei of the brain responsible for autonomic cardiovascular control.

Keywords: Heart failure; digitoxin; baroreflex sensitivity; cardiopulmonary reflex; renal sympathetic nerve activity; spectral analysis; sympathetic vasomotor tone
INTRODUCTION

Heart failure (HF) is a common, chronic, progressive disease and a major cause of mortality and morbidity (Hamdani and Paulus 2011; Mangini et al. 2008). Although the mechanisms underlying the cardiovascular alterations related to HF are well-established, strategies avoiding the progression and outcomes of this disease are limited to classes of drugs used to treat compensatory adjustments following HF, including the activation of the renin-angiotensin-aldosterone system and sympathoexcitation (Coons et al. 2011; Hamdani and Paulus 2011). There is a close relationship between the severity of HF and increased sympathetic vasomotor tone (Carillo et al. 2012).

In chronic HF, the sympathetic drive to the blood vessels of the heart, kidney and muscle blood is dramatically increased and contributes to the progression of the disease (Parati and Esler 2012). Studies have demonstrated a new potential treatment for heart failure with catheter-based renal denervation, which reduces blood pressure and sympathetic drive (Booth et al. 2015). However, the mechanism triggering the increased sympathetic activity at different targets is not well-understood and is a matter of discussion. Increased central sympathetic drive is a reasonable explanation for this phenomenon. The upregulation of the angiotensin II (Ang II) AT1 receptor in conjunction with the downregulation of the AT2 receptor in regions of the brain related to cardiovascular control is present in HF, which would partially explain the increased sympathetic drive associated with this condition (Gao et al. 2008). Impairment of arterial baroreceptor function in HF could be an additional factor leading to increases in sympathetic drive (Han et al. 2010) and a cardiac
autonomic imbalance characterized by sympathetic hyperactivity and reduced vagal tone (Morais et al. 2015).

One of the pharmacological choices to treat HF is digitalis, which improves systolic and diastolic function, as well as the autonomic balance to the heart ("The effect of digoxin on mortality and morbidity in patients with heart failure. The Digitalis Investigation Group" 1997). Digitalis decreases plasma renin activity and consequently alters the levels of Ang II and aldosterone and decreases serum levels of norepinephrine (Gheorghiade and Ferguson 1991). Digitalis inhibits the Na+/K+/ATPase and may promote an increase in arterial baroreceptor sensitivity (Gheorghiade and Ferguson 1991).

However, the mechanisms triggering increased sympathetic activity at different targets in HF and how digitalis decreases sympathetic drive in this condition are not well-understood. The present study aimed to investigate changes in renal sympathetic nerve activity and autonomic cardiovascular function, an investigation that included a spectral analysis of blood pressure and arterial baroreceptor and cardiopulmonary reflex sensitivity, in HF rats chronically treated with digitoxin.

**METHODS**

All experimental procedures were conducted according to the National Institutes of Health Guidelines for the Use and Care of Animals, and the study protocol was approved by the Ethics in Research Committee of the University Federal of São Paulo (process No. 0442/11). Male Wistar rats (initially weighing 200 g) were obtained from the animal care facility of our institution. The animals were housed in group cages, given access to rat chow and water *ad libitum* and
maintained in a temperature-controlled environment (23°C) on a 12-h light/dark cycle.

EXPERIMENTAL PROTOCOLS

The experiments were performed with the following three experimental groups: Sham, HF and HF+DIG. Seven days after surgery to induce myocardial infarction, the rats were submitted to Doppler echocardiography, and the rats with infarctions involving a minimum of 40% of left ventricular cardiac mass were selected and divided into two groups as follows: without treatment (HF) and submitted to digitoxin treatment for 60 consecutive days (HF+DIG). At the end of treatment, the mean arterial pressure (MAP), heart rate (HR), baroreflex control of HR and cardiopulmonary reflex were assessed in conscious rats. The spectral analysis of blood pressure was analysed offline (sample rate 2 KHz). The same group of rats was slowly anesthetized with urethane (1.4 g/kg, i.v.) (Sigma-Aldrich Co, St Louis, MO, USA) to avoid any changes in MAP, and basal renal sympathetic nervous activity (rSNA) and arterial baroreceptor control of rSNA were assessed.

SURGICAL PROCEDURE

After isoflurine-induced anaesthesia (2%), the animals were immobilized in the supine decubitus position. A left thoracotomy was performed in the fourth intercostal space, and two perpendicularly positioned murine retractors separated the ribs. Radiofrequency current (RF) lesions were created with a commercially available, conventional RF generator (model TEB RF10; Tecnologia Eletrônica Brasileira Ltda, São Paulo, Brazil). RF current (1000 KHz) was delivered at constant power (12 watts) for 12 seconds. The power output was automatically shut down if the impedance exceeded 200 ohms. The
damaged area was immediately confirmed by the presence of a clear white, disk-shaped region of coagulation necrosis. Thereafter, the heart was quickly returned to the thorax; pulmonary hyperinsufflation was performed, and a previously made purse string suture was used to close the chest. The above procedures were performed according to a previous report (Antonio et al. 2009).

**DOPPLER ECHOCARDIOGRAPHY AND PULMONARY WATER CONTENT**

Doppler echocardiographic examinations were performed using an HP SONOS 5500 (Philips Medical System, Andover, MA) with a 12-MHz transducer at a depth of 2 cm according to a previous report (Antonio et al. 2009; Cury et al. 2005; Kanashiro et al. 2006). Briefly, seven days after surgery, the rats were anesthetized with ketamine + xylazine, and 2-dimensional and M-mode images from the parasternal longitudinal, transverse, and apical views were obtained and recorded on a 0.5-inch videotape. The image analysis and measurements were performed offline. ECHO detected MI based on subjective identification of akinesis or dyskinesis. The MIS was determined posteriorly in the stained myocardium at the completion of the protocol as described below. The end-diastolic (LVAd) and end-systolic (LVAs) LV transverse areas were measured in the 3 transverse planes (basal, medium, and apical), and LV systolic function was assessed by the change in fractional area (FAC: LVAd - LVAs/LVAd X 100). Diastolic function was assessed by calculating the peak E and A blood flow mitral velocities and the E/A ratio. For this purpose, the sample volume of the pulsed wave Doppler was positioned at the tips of the mitral valve leaflets in an apical 4-chamber view.

Pulmonary water content (%) was obtained based on wet and dry weights. After measuring the right lung wet weight, the tissue was placed in an
oven and maintained at 80°C for 72 hours. After measuring the dry weight of each rat lung, the water content was determined using the following equation:

\[(\text{wet weight and dry weight/wet weight}) \times 100\]

**COMPOSITION AND DIET SUPPLEMENTED WITH DIGITOXIN**

According to a previous report (Helber et al. 2004), the concentration of digitoxin was determined by taking into account the following: (i) orally administered digitoxin is completely absorbed, (ii) a daily dose similar to the one used in the present study was previously shown to be efficient in promoting cardiac benefits in the rat (100 µg/kg per day) when injected subcutaneously, and (iii) young and adult healthy rats in our laboratory eat approximately 30 g of food daily. Digitoxin (1 mg) was diluted in absolute ethyl alcohol (3 mL sprayed in and mixed with standard diet (100 g)). The resultant chow, with digitoxin added and mixed with water, resulted in dough. Chow pellets were made and left to dry for 24 h at 50°C to allow for the complete evaporation of the alcohol.

**ANALYSIS OF CARDIOVASCULAR FUNCTION IN CONSCIOUS RATS**

For the intravenous injection of drugs and direct arterial pressure recording, the rats were anesthetized with ketamine and xylazine (40 and 20 mg/kg, i.p., respectively) (Vetbrands, Jacareí, Brazil) and fitted with femoral venous and arterial catheters. After ≥ 24 h of surgical recovery, MAP and HR were recorded in conscious rats using an analog-digital board PowerLab (ADInstruments, Australia).

For the analysis of the arterial baroreceptor function in conscious rats, bolus injections (0.1 ml) of phenylephrine (3, 5 and 7 µg/Kg, i.v.) or sodium nitroprusside (1, 2 and 3 µg/kg, i.v.) (Sigma-Aldrich Co, St Louis, MO, USA) were administered with ≥ 5-min intervals between doses until blood pressure
returned to baseline. Values of matching MAP variations (ΔMAP from 20 to 55 mmHg) with reflex heart rate (ΔHR) responses were separately plotted for each vasoactive drug to create linear regression curves of baroreceptor function for each group, and their slopes (spikes per second per millimetre of Hg) were compared to analyse the changes in baroreflex sensitivity.

SPECTRAL ANALYSIS OF HEART RATE AND SYSTOLIC BLOOD PRESSURE

For the spectral analysis of heart rate and systolic pressure variability, beat-by-beat HR and systolic arterial pressure data were obtained in conscious rats for the first 10 min of the recording period. The spectral power of the various frequency components of HR and systolic pressure were calculated with fast Fourier transform (FFT) algorithm. This analysis requires data collection at equal time intervals. Therefore, heart rate and systolic pressure were calculated every 100 ms with a cubic spline interpolation (10 Hz). The interpolated series were divided into half-overlapping sequential sets of 512 data points (51.2 s). The segments were inspected visually; non-stationary data were not analysed. A Hanning window was used to attenuate the side effects. The power intensity was computed with a direct FFT algorithm for discrete time series. The total power in the low frequency band (LFb: 0.2-0.75 Hz) and high frequency band (HFb: 0.75-3 Hz) was calculated. The LFb/HFb power ratio was calculated and used as an indicator of cardiac sympathovagal balance (Montano et al. 1994).

CARDIAC AUTONOMIC TONE AND INTRINSIC HEART RATE

Tonic sympathetic and vagal influences to the heart were determined by measuring HR changes after the selective pharmacological blockade of cardiac autonomic receptors. The bradycardic response obtained after a β-adrenergic
receptor blockade with atenolol (1 mg/kg, i.v. - Sigma-Aldrich Co, St Louis, MO, USA) was used to estimate sympathetic tone. The tachycardia response after muscarinic cholinergic receptor blockade with methyl atropine (3 mg/kg, i.v. - Sigma-Aldrich Co, St Louis, MO, USA) was used to estimate vagal tone. The HR in the presence of both autonomic blockers was considered the intrinsic HR.

ANALYSIS OF THE BEZOLD-JARISCH REFLEX SENSITIVITY

In conscious rats, bolus injections (0.1 ml) of phenylbiguanide (PBN) (12.5, 25, 50 µg/Kg) were administered with ≥ 15-min intervals between doses until blood pressure returned to baseline. The percentages of MAP variation (%ΔMAP) and HR variation (%ΔHR) were compared among the groups.

ANALYSIS OF RENAL SYMPATHETIC NERVE ACTIVITY IN URETHANE ANESTHETIZED RATS

Rats were slowly anesthetized with urethane, and the left renal nerve was retroperitoneally exposed and placed on bipolar silver electrodes. When the conditions for nerve recording were established, the nerve and electrode were covered with paraffin oil. The signal from the renal nerve was displayed on an oscilloscope (Tektronix, TDS 220), and nerve activity was amplified (gain 20000, Neurolog, Digitimer, UK), filtered by a band-pass filter (100–1000 Hz), and collected for display and subsequent analysis using a PowerLab data acquisition system (ADInstruments, Australia). At the end of the experiments, the background noise level was determined following hexamethonium bromide (30 mg/kg, i.v.) (Sigma-Aldrich Co, St Louis, MO, USA) administration. The rSNA was rectified online, integrated from the raw data obtained for each heart period, and expressed as volts-seconds (V.s). Additionally, neural activity was analysed offline using the appropriate software (Spike Histogram,
ADInstruments - Australia). The responses of rSNA to the various stimuli are expressed as the percentage of change compared with the basal value obtained immediately before each test. For this purpose, the raw nerve signal was passed through a spike discriminator (PowerLab) to remove background noise, and the total nerve activity, which is expressed in spikes per second (spikes/s), was computed from the time at which the value changed from the basal value to when it returned to the basal value. The basal rSNA is expressed as spikes/s over a period of 60 s. The mean value obtained was compared with the mean value determined before each test as reported previously. Only experiments in which the level of background noise was confirmed at the end of the experiments following hexamethonium and terminal anaesthesia are included in this report.

For the analysis of arterial baroreceptor function sensitivity for rSNA control in anesthetized rats, continuous infusions of phenylephrine (10 µg/Kg, 0.2 mL, 12 mL/h, i.v.) or sodium nitroprusside (20 µg/kg, 0.1 mL, 6 mL/h, i.v.) (Sigma-Aldrich Co, St Louis, MO, USA) were administered with a 1 ml syringe mounted on a syringe pump (model KDS 100, KD Scientific). Values of matching MAP variations (ΔMAP from 20 to 55 mmHg) with reflex renal sympathetic nerve activity (ΔrSNA) responses were separately plotted for each vasoactive drug to create linear regression curves of baroreceptor function for each group, and their slopes (spikes per second per millimetre of Hg) were compared to analyse the changes in baroreflex sensitivity.

STATISTICAL ANALYSES
The results are shown as the mean ± standard error of the mean for functional experiments, and the data of the spectral analyses are shown as the
mean ± standard deviation of the mean. Data were evaluated by one-way ANOVA, followed by the Tukey test. The level of statistical significance was defined as $p<0.05$.

**RESULTS**

**DOPPLER ECHOCARDIOGRAPHY AND PULMONARY WATER CONTENT**

Table 1 shows the echocardiogram data, the other parameters of cardiac remodelling and the pulmonary water content. All rats used in this study had an area of infarction greater than 40%. The HF group showed an increase in systolic area ($0.30$ cm$^2$, $n=9$) and systolic circumference ($1.81$ cm, $n=9$) compared with the Sham group (systolic area: $0.07$ cm$^2$; systolic circumference: $0.86$ cm, $n=5$). In this case, treatment with digitoxin did not improve these parameters (systolic area: $0.41$ cm$^2$; systolic circumference: $2.18$ cm, $n=5$). HF (80.8 ± 0.2%, $n=9$) and the HF+DIG group (80.6 ± 0.1%, $n=5$) presented with an increase in pulmonary water content compared with the Sham group (78.1 ± 0.3%, $n=5$).

**CARDIOVASCULAR AND AUTONOMIC BASAL PARAMETERS**

The HF rats showed decreased MAP ($91 ± 5.0$ mmHg, $n=5$) and HR ($314 ± 9.8$ bpm, $n=8$) compared with the Sham group (MAP: $109 ± 3$ mmHg, $n=5$; HR: $350 ± 12.7$ bpm, $n=6$). Digitoxin treatment did not change these values in the HF+DIG group (MAP: $92 ± 2.1$ mmHg, $n=7$; HR: $296 ± 7$ bpm, $n=9$), as shown in Figure 1A and B. The HF rats presented with increased rSNA ($190 ± 30$ pps, $n=5$) compared with the Sham rats ($98 ± 14.7$ pps, $n=5$). Digitoxin treatment significantly decreased and normalized rSNA in the HF+DIG group ($98 ± 14.3$ pps, $n=7$), as shown in Figure 1C. Figure 1D shows a representative
tracing of the cardiovascular and autonomic parameters in the Sham, HF and 
HF+DIG groups.

**EFFECT OF TREATMENT HEART RATE AND SYSTOLIC PRESSURE VARIABILITY**

A spectral analysis of HR variability (HRV) showed an increase in LFb in 
the HF group (23 ± 5 ms$^2$, $n=8$) compared with the Sham group (14 ± 3 ms$^2$, 
$n=9$); treatment with digitoxin normalized this parameter in the HF+DIG group 
(15 ± 3 ms$^2$, $n=9$). The HF group also showed decreased HFb (77 ± 5 ms$^2$, $n=8$) 
compared with the Sham group (86 ± 3 ms$^2$, $n=9$); digitoxin normalized this 
parameter in the HF+DIG group (85 ± 3 ms$^2$, $n=9$). The results are summarized 
in Table 2.

A spectral analysis of systolic blood pressure variability showed an 
increase in LFb in the HF group (6 ± 1 mmHg$^2$, $n=8$) compared with the Sham 
group (5 ± 2 mmHg$^2$, $n=9$); digitoxin treatment decreased this parameter in the 
HF+DIG group (2 ± 0.3 mmHg$^2$, $n=9$). The HF+DIG group also showed a 
decrease in HFb (1 ± 0.3 mmHg$^2$, $n=9$) compared to HF group (3 ± 0.4 mmHg$^2$, 
$n=8$) and Sham group (2 ± 0.4 mmHg$^2$, $n=9$). The results are summarized in 
Table 2.

**ARTERIAL BAROREFLEX SENSITIVITY:**

A preferential decrease in baroreflex sensitivity for HR control induced by 
phenylephrine (baroreceptor loading) was observed in the HF and HF+DIG 
groups compared with the Sham group, as shown in Figure 2 A, and the 
individual slope values are shown in Table 3. However, no difference among 
the groups was found in relation to baroreceptor sensitivity for rSNA control, as 
shown in Figure 2 B, and the individual slope values are shown in Table 3.
BEZOLD-JARISCH REFLEX:

The HF and HF+DIG groups showed a decrease in %ΔMAP responses in all doses of PBN (HF: 12.5 µg: -17.2 ± 3.6 mmHg; 25 µg: -27.6 ± 3.8 mmHg; 50 µg: -30.3 ± 3.4 mmHg, n=11; HF+DIG: 25 µg: -26.4 ± 4.8 mmHg, n=12) compared with the Sham group (12.5 µg: -34.5 ± 3 mmHg; 25 µg: -47.4 ± 5.6 mmHg; 50 µg: -53.3 ± 4 mmHg, n=7), as shown in Figure 3 A. The HF group also showed a decrease in %ΔHR at intermediate and highest doses of PBN (25 µg: -52.2 ± 3 bpm, n=8 and 50 µg: -56.3 ± 2.5 bpm, n=8) compared with the Sham group (25 µg: -71 ± 2.5 bpm, n=4 and 50 µg: -72.5 ± 2.2 bpm, n= 4), and digitoxin treatment (HF+DIG) promoted a decrease in %ΔHR for the same doses of PBN (25 µg: -40.4 ± 2.8 bpm, n=9 and 50 µg: -40 ± 3.7 bpm, n= 9) compared with the HF group, as shown in Figure 3 B.

DISCUSSION

The major new findings of the present study were as follows: 1) digitoxin treatment prevented increases in rSNA and alterations of the autonomic balance to the heart and blood vessels in the HF-treated group (HF+DIG) and 2) these improvements in cardiovascular function were independent of cardiac remodelling and peripheral cardiovascular reflexes, including the arterial baroreceptor and cardiopulmonary reflexes. The mechanisms underlying sympathoexcitation in HF are not well-understood. Some hypotheses suggest that reflex sympathoexcitation is a consequence of decreased blood pressure and increased circulating and tissue
Ang II (DiBona et al. 1995) or that sympathoexcitation occurs even in response to a decrease in arterial baroreflex sensitivity. These processes may result in a reduction in the inhibitory influence on afferent vasomotor centres in the brain, resulting in neuro-humoral efferent sympathetic outflow (Ferguson et al. 1984b).

Considering that increased sympathetic activity to the kidneys leads to important changes in kidney function, including renin secretion, vasoconstriction and sodium reabsorption (DiBona et al. 1995) and that all of these changes are present in HF, new strategies to control autonomic dysfunction in HF are important for the outcome of the disease.

In the present study, we observed a preferential decrease in baroreceptor reflex sensitivity for HR but not for rSNA in the HF rats compared with the Sham group. These data suggest that the increased rSNA in the HF group was not directly related to changes in baroreceptor reflex sensitivity, as previously reported (Ferguson et al. 1984a).

The decrease in MAP in the HF rats could trigger other compensatory mechanisms, such as renin-angiotensin-aldosterone system activation, which could contribute to increases in rSNA, as proposed previously (Dibona et al. 1995). However, in the present study, a significant decrease in rSNA was found in the HF+DIG group, suggesting that sympathoexcitation is not related to low blood pressure because the HF+DIG and HF groups had similar blood pressure levels. Changes in the central nuclei in the brain related to cardiovascular control could be involved in the increases in rSNA; we also noted an imbalance in the autonomic control of the heart with an increase in LFb and a decrease in HFb in the HF rats. Wang et al. (Wang et al. 2009) found that microinjections of a glutamate receptor antagonist into the rostralventrolateral medulla (RVLM) of
HF rats significantly decreased MAP and rSNA, suggesting that increased glutamate receptor expression in the RVLM contributes to increased sympathetic vasomotor drive in HF.

However, there is controversy regarding baroreceptor dysfunction in HF. The divergence is related to the animal model used for HF induction, the extension of myocardial infarction and the time after myocardial infarction at which the experiments were performed. According to Ramchandra et al. (Ramchandra et al. 2014), baroreflex control for HR and rSNA, but not for cardiac sympathetic nerve activity (cSNA), was impaired in sheep with HF. However, Zucker et al. (Zucker et al. 1985) reported that arterial baroreceptor sensitivity for rSNA in dogs with HF was normal compared with control animals. This controversy is not limited only to HF animal models. Floras et al. (Floras, 2001) found that humans with advanced HF had decreased baroreceptor reflex sensitivity relative to HR control but not to muscle ANS compared with healthy subjects. Others (Dibnerdunlap and Thames 1989; Grassi et al. 1995) noted decreased sensitivity of baroreceptor reflex responses to muscle sympathetic nerve activity in humans with HF.

Together, these studies suggest a territory-dependent difference in baroreceptor reflex sensitivity in HF in different species. The same was found in the present study: a decrease was noted in the sensitivity of the baroreceptor reflex for HR control but not for of rSNA control.

However, this was the first study to investigate baroreceptor function in a model of HF induced by ablation. The ablation model of HF was chosen due to its low mortality rates and its low variability in the size of the infarct area among animals. In addition, this model does not cause papillary muscle injury, as noted
in the occlusion model, and thus does not lead to changes in cardiac valves that could hamper the interpretation of the results. In addition, as presented in Table 1 (echocardiography parameters), this model has specific characteristics of HF, as described in other models of HF animals (dos Santos et al. 2008; Moisés et al. 2000).

We noted significant impairment of the cardiopulmonary reflex in HF animals; a similar finding was previously reported (Modesti et al. 2004). Ferguson et al. (Ferguson et al. 1984b) found that the decreased sensitivity of the cardiopulmonary and arterial baroreceptor reflexes may result in decreased afferent inhibitory influence on the vasomotor centres of the brain, resulting in increased sympathetic efferent and neurohumoral excitation. Thus, we can infer that the reduction in the sensitivity of the cardiopulmonary reflex is one of the factors that may trigger increased rSNA. However, digitoxin treatment significantly reduced rSNA and caused further impairment of the cardiopulmonary reflex, suggesting that rSNA reduction induced by digitoxin in HF rats is not directly related to the cardiopulmonary reflex.

Digitoxin sensitizes the vagal afferents mediating the cardiopulmonary reflex, leading to a reduction in rSNA in dogs (Thames et al. 1982). These findings could have implications concerning the potential mechanism of action of digitalis in heart failure. However, in the present study, there was no correlation between the sympathoinhibition induced by digitoxin and the cardiopulmonary reflex. Therefore, the improvement in survival induced by digitoxin in HF rats could be related to rSNA reduction (Helber and Tucci 2010).

Another interesting result was that the animals treated with digitoxin showed no improvement in cardiac remodelling, as shown by the
echocardiographic data in Table 1. However, there was improvement and normalization of autonomic balance to the heart and blood vessels, as shown by the HRV results in Table 2. These results demonstrate that digitoxin has cardioprotective effects and promotes the reduction of sympathetic vasomotor drive. Moreover, we believe that digitalis influences autonomic modulation, most likely through central actions in the brain. In addition, digitoxin may act directly on the sinus node, improving the autonomic balance to the heart because digitoxin acts on the Na⁺/K⁺/ATPase, which is present in all cells. In excitable tissues, its activity is essential for regulating membrane potential and is responsible for cellular excitability (Teruya et al. 1997). However, the relative importance of the central versus peripheral actions of digitoxin is still a matter of discussion.

Although digitoxin has a very low therapeutic range and can be extremely toxic, we used a study previously conducted in our laboratory as a reference (Helber, et al. 2004) as this study described the importance of serum digitoxin and the mortality rates of infarcted animals. Furthermore, according to Wasserstrom and Aistrup (2005), rats are less sensitive to cardiac glycoside due to resistance in alpha isoform 1; therefore, the dose used in this study was higher than that used in humans but was not toxic to the animals (Helber, et al. 2004).

Conclusion:

In the present study, digitoxin reduced and normalized rSNA and improved the autonomic balance to the heart in HF rats; similar data have been previously described (Gheorghiade and Ferguson 1991; Mason and Braunwald 1964). However, the mechanism underlying the sympathoinhibition induced by
digitoxin remains unknown. Because the HF animals treated with digitoxin showed no improvement in the sensitivities of their arterial and cardiopulmonary baroreceptor reflexes, it is possible that digitoxin acts in the brain to reduce sympathetic vasomotor drive to the kidneys and heart.

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DISCLOSURE

The authors declare no conflicts of interest.

References


Table 1. Doppler echocardiographic parameters for the Sham, Heart Failure (HF) and HF treated with digitoxin (HF+DIG) groups. Values are the mean ± standard error of the mean. *p<0.05 compared with the Sham group. One-way ANOVA followed by Tukey post-test.

Figure 1. (A) Mean Arterial Pressure (MAP). (B) Heart Rate (HR). (C) renal Sympathetic Nerve Activity (rSNA). (D) Typical tracings of MAP, HR and rSNA. Sham and Heart Failure (HF) untreated and treated (HF+DIG). Values are the mean ± standard error of the mean. *p<0.05 compared with the Sham group; #p<0.05 compared with the Heart Failure (HF) group. One-way ANOVA followed by Tukey post-test.

Table 2. Blood pressure and pulse interval variability for the Sham, Heart Failure (HF) and HF treated with digitoxin (HF+DIG) groups. Values were compared and are presented as the mean ± standard deviation. ANOVA test followed by Tukey post-test. *p<0.05 vs Sham and #p<0.05 vs HF.
Abbreviations: LFb = Low frequency band, HFb = High frequency band.

Figure 2. (A) Baroreceptor reflex function for heart rate (HR) control. Linear regression graph containing (1) reflex increases in HR in response to decreases in mean arterial pressure (MAP) produced by bolus injections of (0.1 ml) sodium nitroprusside (1, 2 and 3 µg/kg, i.v.) and (2) reflex decreases in HR in response to increased MAP produced by bolus injections of (0.1 ml).
phenylephrine (3, 5 and 7 µg/Kg, i.v.). (B) Baroreceptor reflex function for renal sympathetic nerve activity (rSNA) control. Nonlinear regression graph containing (1) reflex increases in rSNA in response to decreases in mean arterial pressure (MAP) produced by continuous infusions of sodium nitroprusside (20 µg/kg, 0.1 mL, 6 mL/h, i.v.) and (2) reflex decreases in rSNA in response to increased MAP produced by continuous infusions of phenylephrine (10 µg/Kg, 0.2 mL, 12 mL/h, i.v.). Values are the mean ± standard error of the mean. *p<0.05 compared with the Sham group before digitoxin; and #p<0.05 compared with the Heart Failure (HF) group before digitoxin.*p<0.05 compared with the Sham group. One-way ANOVA followed by Tukey post-test.

Table 3. Slope values obtained from the linear and nonlinear regression lines of the baroreceptor reflex function control for heart rate (HR) and of the renal sympathetic nerve activity (rSNA), respectively, for the Sham, Heart Failure (HF) and HF treated with digitoxin (HF+DIG) groups. Values are the mean ± standard error of the mean. *p<0.05 compared with the Sham group. One-way ANOVA followed by Tukey post-test.

Figure 3. Bezold-Jarisch reflex. (A) Percentage of mean arterial pressure variation (%ΔMAP) induced by bolus injection (0.1 ml) of phenylbiguanide (PBN) (12.5, 25, 50 µg/Kg). (B) Percentage of heart rate variation (%ΔHR) induced by bolus injection (0.1 ml) of phenylbiguanide (PBN) (12.5, 25, 50 µg/Kg). Values are the mean ± standard error of the mean. *p<0.05 compared with the same dose of phenylbiguanide (PBN) in the Sham group; #p<0.05 compared with the
same dose of PBN in the Heart Failure (HF) group. One-way ANOVA followed by Tukey post-test.
A  Baroreflex sensitivity of heart rate

B  Baroreflex sensitivity of renal sympathetic nerve activity
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<thead>
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<th>Parameters</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham (N= 5)</td>
</tr>
<tr>
<td>Myocardial Infarction Size</td>
<td>0%</td>
</tr>
<tr>
<td>Pulmonary water content</td>
<td>78.1 ± 0.3%</td>
</tr>
<tr>
<td>Fractional Shortening of the Transverse Area</td>
<td>0.73 ± 0.008%</td>
</tr>
<tr>
<td>Systolic area</td>
<td>0.07 ± 0.006 cm²</td>
</tr>
<tr>
<td>Systolic circumference</td>
<td>0.86 ± 0.1 cm</td>
</tr>
</tbody>
</table>

Table 1. Doppler echocardiographic parameters for the Sham, Heart Failure (HF) and HF treated with digitoxin (HF+DIG) groups. Values are the mean ± standard error of the mean. *p<0.05 compared with the Sham group. One-way ANOVA followed by Tukey post-test.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham (N= 9)</td>
</tr>
<tr>
<td><strong>Pulse Interval</strong></td>
<td></td>
</tr>
<tr>
<td>LFb (ms²)</td>
<td>14 ± 3</td>
</tr>
<tr>
<td>HFB (ms²)</td>
<td>86 ± 3</td>
</tr>
<tr>
<td>LFb/HFB</td>
<td>0.2 ± 0.05</td>
</tr>
<tr>
<td><strong>Systolic Blood Pressure</strong></td>
<td></td>
</tr>
<tr>
<td>LFb (mmHg²)</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>HFB (mmHg²)</td>
<td>2 ± 0.4</td>
</tr>
</tbody>
</table>

Table 2. Blood pressure and pulse interval variability for the Sham, Heart Failure (HF) and HF treated with digitoxin (HF+DIG) groups. Values were compared and are presented as the mean ± standard deviation. ANOVA test followed by Tukey post-test. *p<0.05 vs Sham and #p<0.05 vs HF. LFb = Low frequency band, HFB = High frequency band.
### Reflex responses for heart rate

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pressor response</th>
<th>Depressor response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\text{Slope (pps/mmHg)})</td>
<td>(\text{Slope (pps/mmHg)})</td>
</tr>
<tr>
<td>Sham</td>
<td>-2.27 ± 0.23, N= 6</td>
<td>-3.17 ± 0.32, N= 6</td>
</tr>
<tr>
<td>HF</td>
<td>-1.24 ± 0.07, N= 8*</td>
<td>-3.54 ± 0.27, N= 8</td>
</tr>
<tr>
<td>HF+DIG</td>
<td>-1.18 ± 0.09, N= 9*</td>
<td>-2.86 ± 0.25, N= 9</td>
</tr>
</tbody>
</table>

### Reflex responses of renal sympathetic nerve activity

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pressor response</th>
<th>Depressor response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\text{Slope (pps/mmHg)})</td>
<td>(\text{Slope (pps/mmHg)})</td>
</tr>
<tr>
<td>Sham</td>
<td>-1.94 ± 0.45, N= 5</td>
<td>-0.82 ± 0.15, N= 5</td>
</tr>
<tr>
<td>HF</td>
<td>-2.62 ± 0.30, N= 7</td>
<td>-0.81 ± 0.35, N= 5</td>
</tr>
<tr>
<td>HF+DIG</td>
<td>-1.63 ± 0.49, N= 7</td>
<td>-0.80 ± 0.08, N= 7</td>
</tr>
</tbody>
</table>

**Table 3.** Slope values obtained from the linear regression line of the baroreceptor reflex function control for heart rate (HR) and renal sympathetic nerve activity (rSNA) for the Sham, Heart Failure (HF) and HF treated with digitoxin (HF+DIG) groups. Values are the mean ± standard error of the mean. *p<0.05 compared with the Sham group. One-way ANOVA followed by Tukey post-test.