Regulatory Peptides 181 (2013) 30–36

Contents lists available at SciVerse ScienceDirect

Regulatory Peptides

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

Cardiovascular and eletrocardiographic parameters after tonin administration in Wistar rats

Denis D. Damasceno [a,b,*], Mercia P. Lima [c], Daisy F. Motta [b], Anderson J. Ferreira [d], Judson F. Quintão-Junior [e], Lucas R. Drummond [e], Antônio J. Natali [e], Alvair P. Almeida [b], Jorge L. Pesquero [b]

[a] Department of Educational Development, Federal Institute of Education, Science and Technology of Southeast Minas Gerais, Campus Barbacena, MG, Brazil
[b] Department of Physiology and Biophysics, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil
[c] Nursing School, Federal University of Minas Gerais, Belo Horizonte, Brazil
[d] Department of Morphology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil
[e] Department of Physical Education, Biological Sciences and Health Center, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil

ARTICLE INFO

Article history:
Received 15 April 2012
Received in revised form 12 October 2012
Accepted 17 December 2012
Available online 11 January 2013

Keywords:
Tonin
Renin–angiotensin system
Isolated heart
Electrocardiogram
Autonomic tonus

ABSTRACT

In order to understand the mechanisms of interaction between tonin–angiotensin and renin–angiotensin systems (RAS) we evaluated, "in vivo" and "in vitro", in Wistar rats, cardiovascular and electrocardiographic parameters after tonin administration. Arterial pressure (AP) and electrocardiogram (ECG) were recorded in awake animals before and after tonin administration. Langendorff technique was used to analyze cardiac function in isolated heart in the presence of tonin and video motion edge detection system was used to evaluate the effect of tonin upon contractile function of isolated rat ventricular cardiomyocytes. After tonin infusion rats presented significantly higher diastolic and mean arterial pressure (MAP) and heart rate (HR) as compared with control. The ECG analysis revealed shorter RR interval, increase in the low-frequency (LF) range of the heart rate variability (HRV) power (%) and decrease in the high-frequency (HF) of HRV power (%). Isolated hearts perfused with tonin presented an increase in the arterial coronary pressure (ACP) and decline in the ventricular systolic tension (ST), maximal ($dT/dt+$) and minimal (dT/dt) contractility. The rates of contraction and relaxation of isolated ventricular cardiomyocytes were significantly increased due to the presence of tonin. The angiotensin II (Ang II) levels in the coronary sinus effluent increased in the presence of tonin in a dose-dependent manner and the effect of tonin upon ACP was completely blocked by candesartan. Tonin is able to generate the vasoconstrictor peptide Ang II in the isolated heart of the rat and the cardiovascular response induced by tonin was completely blocked by candesartan, an indication that the action of Ang II on Ang II type 1 (AT1) receptors is the major mechanism of the heart effects. Tonin affects cardiomyocyte contractile function which may be due to interference with Ca^{2+} handling.

1. Introduction

The renin-angiotensin system (RAS) is well known for its regulation of blood pressure (BP) and fluid homeostasis. The RAS participates in changes in chronotropism, inotropism, arrhythmogenesis and cellular growth in the heart [1]. Ang II, the effector of the system causes vasoconstriction directly and indirectly by stimulating angiotensin II (Ang II) type 1 (AT1) receptors present on the vasculature and by increasing sympathetic tone [2]. All components of the RAS can be found in the heart, vasculature, kidney, among others [3]. Even though some authors speculate on the putative mechanism of renin uptake from the plasma into the heart, it is well described that renin synthesis in the heart is approximately 2% of that of the kidney [4].

The existence of alternative Ang II generating systems in different sites of cardiovascular importance suggests diverse physiological effects for these systems in the local control of blood flow and in the pathophysiology of hypertension and cardiovascular diseases [5]. These systems, involving proteinases other than renin and the angiotensin converting enzyme (ACE), have been shown in several species including human [6–8].

An enzyme that represents a good candidate to be involved in the Ang II generation as an alternative route is the serine proteinase tonin, which is able to release Ang II directly from angiotensinogen (AG) [9]. Tonin is present in many organs of the rat, such as the heart, brain, prostate and submandibular gland [10–12]. Tonin may also release Ang II from peptides presenting the sequence corresponding to the N-terminal portion of AG including angiotensin I (Ang I) and the tetradecapeptide (AG(1–14)) [7], the synthetic renin substrate. The

* Corresponding author at: Department of Educational Development, Rua Monsenhor José Augusto, 224, São José, 36.205-018, Barbacena, MG, Brazil. Tel.: +55 32 3693 8625; fax: +55 32 3693 8614.
E-mail address: denis.damasceno@ifsudestemg.edu.br (D.D. Damasceno).

0167-0115/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.regpep.2012.12.009

observed specificity of the enzyme supports a possible important role of tonin for local Ang II liberation in tissues [13].

In 2003, Borges [12] and collaborators presented evidences that tonin may participate in the process of cardiac hypertrophy in the rat. These authors showed that Wistar rats with isoproterenol-induced cardiac hypertrophy presented increased levels of tonin activity and expression of tonin and AG in the atria. No change was observed in the levels of expression and activity of ACE or renin in cardiac tissue of these animals when compared with the control group. Therefore, to advance in the characterization of the physiological role of tonin more recently our group generates transgenic mice that express rat tonin (TGM(rTon)). These mice present high levels of tonin mRNA and activity specifically in the brain. As a consequence, TGM(rTon) develop increased BP and water intake [14].

In order to understand the mechanisms of interaction between tonin–angiotensin and RAS in the heart, we evaluated "in vivo" and "in vitro" cardiovascular and electrocardiographic parameters of male Wistar rats after tonin administration. Isolated working hearts were used to separate the effects of the local and systemic RAS. In addition, the effect of tonin upon contractile function of isolated rat cardiomyocytes was analyzed.

2. Experimental procedures

2.1. Animals

Experiments were performed in male Wistar rats (250–300 g) from the main breeding stock of the Institute of Biological Sciences (Federal University of Minas Gerais, Brazil). Animals were housed individually in plastic cages, under controlled light conditions (lights on at 6:00 am and off at 8:00 pm), room temperature at 24 °C with food and water ad libitum. Efforts were made to avoid any unnecessary distress to the animals and all animal procedures were performed in accordance with institutional guidelines approved by the Ethics Committee in Animal Experimentation of the Federal University of Minas Gerais, Brazil (CETEA-UFMG), which are in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals. The animals were randomly divided into 3 groups: a) in vivo group—BP, ECG and HR measurements, b) in vitro group—for systolic and diastolic tension analysis in isolated hearts and c) *cardiomyocytes* group, for contractile function determination in isolated ventricular myocytes.

2.2. Blood pressure

Twenty-four hours before the experiment the animals were anesthetized with 2.5% tribromoethanol (1 mL/100 g of body weight, i.p., Sigma-Aldrich, Inc.) and polyethylene catheters (PE-10 connected to PE-50) were inserted into abdominal aorta through the left femoral artery and into the femoral vein for recording of BP and drug infusion, respectively. The catheters were tunneled subcutaneously and exteriorized backing the cervical region of the animal. Five Wistar rats were used for BP measurements. Highly purified tonin [10] was injected into the femoral catheter. MAP and systolic and diastolic arterial pressure (SAP and DAP, respectively) were monitored simultaneously during experiments by a solid-state strain gauge transducer (TSD 104A, Biopac Systems, Inc., CA, USA). The transducer was connected to a computer through a data acquisition system (MP100; Biopac Systems, Inc., CA, USA). The data were analyzed by the AcqKnowledge Software. The experiments were conducted in conscious and freely moving rats.

2.3. Electrocardiogram and heart rate variability

To obtain ECG tracings five Wistar rats were anesthetized with 2.5% tribromoethanol (1 mL/100 g of body weight, i.p., Sigma-Aldrich, Inc.) and a bipolar platinum electrode was positioned in the thorax (subcutaneous tissue) directly in derivation DII [15]. The ECG recordings were performed 24 h after the implantation of the electrode and catheters. Highly purified tonin [10] was injected into the femoral catheter. ECG was evaluated in conscious, freely moving rats. The RR and PR intervals, QT interval duration, corrected QT (QTc) and the QRS complex were analyzed. The QT interval was measured starting from the onset of the QRS complex until the end of the T wave, which is the return of the T wave to the baseline. QTc was obtained using Bazett's formula ($QTc = QT/\sqrt{RR}$) [16]. The HRV was calculated by Kubios software from successive RR interval obtained from the ECG tracings. In the time-domain analysis, the following indexes were evaluated: standard deviation of RR intervals (SDNN) and square root of the mean squared differences of successive RR intervals (RMSSD). Additionally, in the frequency-domain analysis of HRV the LF power (%) and the HF power (%) were evaluated [17].

2.4. Isolated hearts

Thirty five Wistar rats were used for cardiac function evaluation. The hearts were isolated and perfused (8.0 ± 0.2 mL/min) as previously described [18]. All experiments were initiated after a 10-min stabilization period. The drugs were dissolved in KRS (perfusion fluid containing: 118.4 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$–$7H_2O$, 2.5 mM $CaCl_2$–H_2O, 11.7 mM glucose and 26.5 mM $NaHCO_3$) to obtain the desired final concentration. Highly purified tonin [10] was added to the perfusing KRS to 8, 16, 32 and 64 ng/mL and administered to each group separately. The dose of 32 ng/mL was administered in the presence or not of 1 nM candesartan. In the doses of 16 and 64 ng/mL, the coronary sinus effluent was collected (at 0, 5, 10, 15, 20, 25 and 30 min) and subsequently assayed for Ang II by specific radioimmunoassay [19]. Mechanical parameters such as systolic and diastolic tensions and arterial coronary pressure (ACP) were measured throughout the experiment. A force transducer (model FT3, Grass) was attached through a heart clip to the apex of the ventricles to record the contractile force (tension, g) in a computer using a data acquisition system (Biopac System, Inc., CA, USA). A diastolic tension of 1.0 ± 0.1 g was applied to the hearts [20] and we also evaluated the tension developed by the hearts by calculating the derivatives ($\pm dT/dt$) using the software AcqKnowledge Version 3.5.7 (Biopac System, Inc., CA, USA). Maximum dT/dt is a reasonable index of the initial velocity of myocardial contraction and minimum dT/dt with myocardial relaxation.

2.5. Isolated cardiomyocyte

Left ventricular myocytes were isolated as previously described [21]. Cell contractile function was evaluated as previously described [21]. Briefly, isolated cells were placed in a chamber with a glass coverslip base mounted on the stage of an inverted microscope (Nikon Eclipse TS100). Cells were perfused with HEPES Tyrode's solution containing 1 mM $CaCl_2$ plus 0, 10, 15 or 20 ng/mL of tonin, and field stimulated at the frequency of 1 Hz (20 V, 5 ms duration square pulses) via platinum bath electrodes (MyoPacer field stimulator; IonOptix, Milton, MA). Cells were visualized on a personal computer monitor with a NTSC camera (MyoCam; IonOptix) in partial scanning mode. This image was used to measure cell shortening (our index of contractility) in response to electrical stimulation using a video motion edge detector (IonWizard; IonOptix). All parameters were evaluated using customized software developed in the MatLab® platform. Cell shortening from stimulation (expressed as a percentage of resting cell length), time to peak of shortening and time to half relaxation were measured and calculated as previously described [22]. We evaluated 32–38 cells per animal in each experimental group. Only calcium-tolerant, quiescent, rod-shaped cardiomyocytes showing clear cross striations were studied.

2.6. Statistical analysis

Statistical analysis was performed using Graph-Pad Prism 4 (GraphPad Software, Inc.). Data are reported as mean ± SEM. Significant