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Short communication

Chemoreflex control of the cardiovascular system remains altered after recovery from low protein diet early in life

R.W.M. Sá [1], G.S.M. Borges [1], D.A. Chianca Jr., L.B. De Oliveira, L.M. Cardoso *

Federal University of Ouro Preto, Department of Biological Sciences/NUPEB Campus Universitário Morro do Cruzeiro, Ouro Preto, MG 35.400-000, Brazil

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ABSTRACT

This study aimed to evaluate the cardiovascular component of the arterial chemoreflex in rats recovered from low protein diet. Male Fischer rats were randomly divided into control and recovered (R-PR) groups after weaning. R-PR rats were fed with low protein diet for 35 days and recovered under normal protein diet for 70 days. Control rats received normal protein diet for 105 days. Arterial chemoreflex was elicited by intravenous injection of KCN. Results showed that pressor response of the chemoreflex was increased in R-PR. Data suggest that protein restriction may alter cardiovascular response to chemical activation of the chemoreflex after recovery.

Cardiovascular diseases (CVD) are among the most common causes of mortality in the world. Among environmental factors contributing to CVD is the reduced protein intake. A reduction in protein diet leads to changes in cardiovascular homeostasis, affecting peripheral vascular resistance, renin secretion, renal hemodynamics and central neurotransmission of cardiovascular reflex pathways (Langley-Evans, 2001). The chemoreflex is an important regulator of blood pressure. Chemoreflex activation by cytotoxic chemicals or hypoxic hypoxia activates sympathoexcitatory and parasympathoexcitatory efferent drives promoting pressor and bradycardic responses, respectively. Chemoreflex control of the blood pressure is also known by its involvement with hypertension, especially because chronic chemoreflex activation could lead to sustained rise in mean arterial pressure (Fletcher, 2000). Data from literature show that cardiovascular component of chemoreflex is highly responsive in rats that underwent protein restriction early in life and may contribute to keep blood pressure within normal range in these animals (Penitente et al., 2007). The hypothesis that diet recovery could return cardiovascular response to chemoreflex activation back to normal never was properly addressed and tested. Therefore, this short study aimed to evaluate cardiovascular responses to chemoreflex chemical activation in rats recovered from low protein diet to address whether residual changes in the chemoreflex persisted after recovery.

Male Fischer rats were randomly divided into recovered (R-PR) and control groups. R-PR rats were fed with low protein (6% protein, as casein) after weaning period (21 days after birth) for 35 days and recovered under normal protein diet (20% protein) for more 70 days. Control group received only normal protein diet for 105 days. Diets were made isocaloric by replacing protein content for carbohydrate (Tropia et al., 2001). Animals were kept in collective cages, under a 12-hour light/dark cycle and had free access to food and water. Room temperature was kept between 21 and 25 °C. All procedures had the approval of the institutional animal care and use committee of the Federal University of Ouro Preto (protocol no. CEUA 2010/29) and were carried out according to EU Directive 2010/63/EU for animal experiments. Body weight and food intake were monitored during the whole treatment period. Under ketamine (80 mg/kg) plus xylazine (7 mg/kg) anesthesia, polyethylene catheters were inserted into the left femoral artery and vein for measurement of pulsatile arterial pressure (PAP) and drug administration, respectively. The free ends of the catheters were tunneled subcutaneously and exteriorized at the back of the neck. During experiments, arterial cannula was connected to a swivel and to a MLT0699 disposable blood pressure transducer (ADInstruments Pty Ltd, Australia). The analogical signal from the blood pressure transducer was preamplified by a ML 221 Bridge Amp (ADInstruments Pty Ltd, Australia) and digitized by an analog-to-digital converter PowerLab 4/35 (ADInstruments Pty Ltd, Australia). Data were collected at a 1000 Hz sampling rate and a 20 mV range digitizing window. Heart rate (HR) and mean arterial pressure (MAP) were derived on-line from pulsatile arterial pressure signal with LabChart 7.0 for Windows software. All experiments were performed in unanesthetized freely moving rats, approximately 48 h after catheter insertion surgery. Animals were housed in the experimentation room for 48 h before the experiments in order to allow full acclimation to experimental environment. Potassium cyanide (KCN) was obtained from Synth. Fresh made solutions (one for each dose) of KCN were

* Corresponding author at: Campus Universitário Morro do Cruzeiro, ICEB/NUPEB/DECBI, Ouro Preto, MG 35.400-000, Brazil.
E-mail address: leomcardoso@gmail.com (L.M. Cardoso).
[1] Undergraduate students.

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prepared with PBS (phosphate buffered saline) pH 7.3 so that the volume injected for each KCN dose ranged from 0.1 to 0.2 mL. Baseline levels of mean arterial pressure (MAP) and heart rate (HR) were continuously recorded for 30 min after a 15 minute adaptation period. Arterial chemoreflex was elicited with intravenous (*i.v.*) injections of KCN according to previous studies which demonstrated that KCN is a potent activator of peripheral chemoreceptors in rats (Franchini and Krieger, 1993). Different doses of KCN (20, 40, 60, 80 e 160 μg/kg of body weight) were randomly injected *i.v.* at 5 minute interval. Data were expressed as mean ± standard error of the mean (SEM). Baseline levels of MAP and HR were extracted from continuous recording (30 min) of each animal and averaged together to express group data. Cardiovascular responses to arterial chemoreflex activation were quantified as maximal change in MAP and HR from baseline as a result of KCN injections. Average changes in MAP and HR for each KCN dose in control and R-PR groups were analyzed by two way ANOVA followed by Bonferroni's pair wise test. Systolic interval (SI) and systolic blood pressure (SP) variability in the frequency domain were assessed by spectral analysis. A 30 minute period of continuous blood pressure recording was taken from the baseline recordings for spectral and spontaneous baroreflex analysis. Data from sequences of consecutive heartbeats were analyzed by the Cardioseries software v2.3. Both time series were resampled to 10 Hz by cubic spline interpolation and divided into contiguous segments of 512 values, which overlapped by 50%. After Hanning windowing, the spectrum of each segment of either the SP or SI series was calculated using a fast Fourier transform (FFT) algorithm. Two oscillatory components were then compared between groups: low frequency band (LF; 0.20 to 0.75 Hz) and high frequency band (HF; 0.75 to 3.00 Hz). The peak of power density found in LF and HF bands from the spectra were averaged together in each group. Grouped data was compared using unpaired Student *t*-test. The relation LF/HF was also evaluated. The power density of HF and LF bands per se and the relationship between them has been widely used in the literature as a parameter to infer about sympathetic/parasympathetic relationship (autonomic balance) that governs heart rate (Goldstein et al., 2011). Spontaneous baroreflex sensitivity was computed using the sequence method, described by Parati et al (Parati et al., 1988) using the Cardioseries software v2.3. Briefly, the method is based on the identification of three or more consecutive beats in which progressive increases/decreases in systolic blood pressure are followed by progressive lengthening/shortening in systolic interval. Linear regressions of individual data are performed to assess slopes and all computed slopes were averaged together to obtain mean spontaneous baroreflex sensitivity (BRS). Data sets were considered statistically different when p value (probability of type I error) was smaller than 0.05. All statistical analyses were performed by GraphPad Prism 6.02 for Windows.

After weaning, on the first day of treatment, control and R-PR groups had 62.4 ± 3.4 g and 78.9 ± 6.8 g respectively. After 35 days (end of the protein restriction period) control and R-PR weighed 184.3 ± 7.3 and 68.7 ± 4.4 g respectively ($p < 0.05$, two way ANOVA). At this point, R-PR rats had no body mass gain and weighed 62.7% less than control rats on average. Through this period, growth slope calculated by linear regression was smaller ($p < 0.0001$) for R-PR group (−0.2 ± 0.11 g/day) compared to control group (3.5 ± 0.14 g/day). After 5 days (40th day) of access to normal protein diet, R-PR rats started a rapid increase in body weight and body gain (28.41 ± 1.26 g for R-PR against 13.42 ± 1.15 g for control on day 50), reaching body weight values close to control rats by day 85. Growth slope for this period was higher ($p < 0.0001$) for R-PR (4.1 ± 0.24 g/day) compared to control (1.9 ± 0.20 g/day). At the end of the treatment period, control and R-PR groups had no significant differences on their body weights (310 ± 13 g for control and 291 ± 16 g for R-PR) as well as in the body weight changes over time (growth slope 1.0 ± 1.10 g/day for control and 1.9 ± 1.46 g/day for R-PR, $p = 0.6383$). Average food intake was similar between groups throughout the entire experimental protocol. No significant differences were observed for MAP (109.3 ± 10 mm Hg for control, n = 7 versus 114.6 ± 3.9 mm Hg for R-PR, n = 5) and HR (332.5 ± 16.3 bpm for control versus 368.4 ± 12.6 bpm for R-PR) between groups. KCN produced transient pressor and bradycardic effects in a dose–response manner as previously described in the literature (Franchini and Krieger, 1993). The results summarized in Fig. 1 showed that pressor responses elicited by KCN injections were higher for the doses 60 μg/kg (23.5 ± 3.6 Δmm Hg for control versus 37.6 ± 5.2 Δmm Hg for R-PR) and 160 μg/kg (36.9 ± 2.8 Δmm Hg for control versus 52.5 ± 4.2 Δmm Hg for R-PR) in R-PR rats compared to control rats. No significant differences were found by two way ANOVA for bradycardic responses between groups. Data from spectral analysis revealed no differences for LF and HF oscillatory components between control and R-PR rats. In addition, no difference was found between groups in the LF/HF ratio. Spontaneous baroreflex sensitivity was 44.12% higher in R-PR (0.49 ±

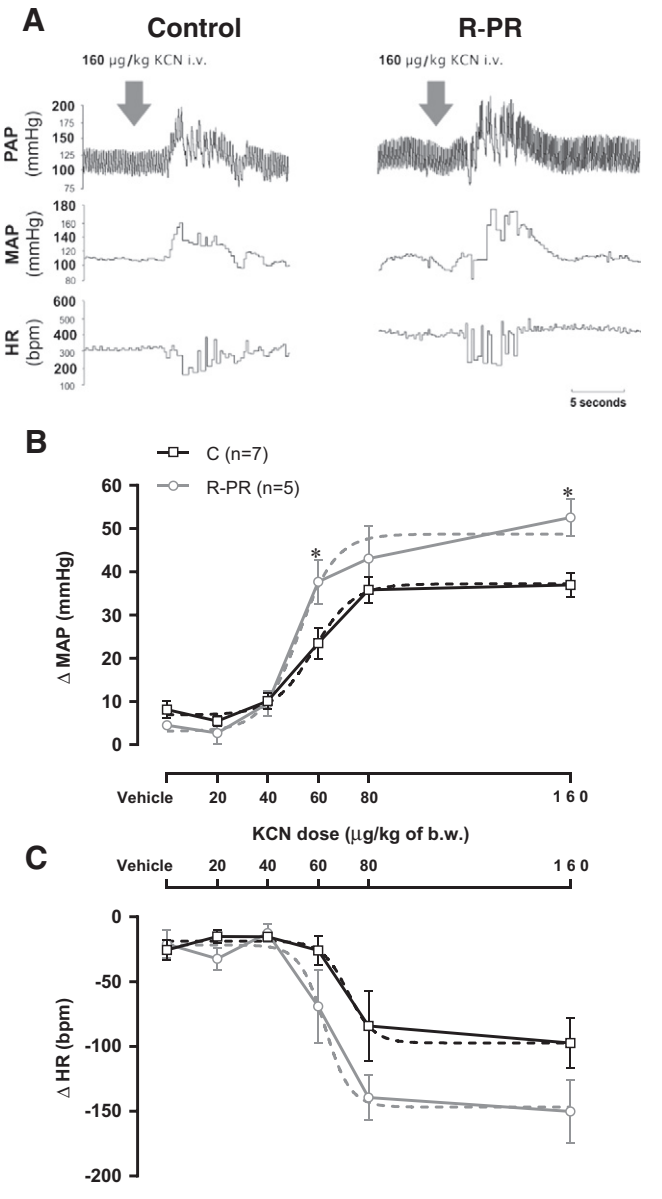

Fig. 1. (A) Traces of pulsatile, mean arterial pressure (PAP and MAP) and heart rate (HR) showing the profile of the cardiovascular responses elicited by intravenous (*i.v.*) injection of KCN (160 μg/kg of body weight) in a control and R-PR rat. Top arrows indicate the exact moment of the injection. Changes in MAP (B) and HR (C) elicited by activation of arterial chemoreflex with different doses of KCN. Dashed lines represent the best fit of the nonlinear regression (4 parameters) for each group. *Different from control; Two way ANOVA followed by Bonferroni's post-test.