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Malnutrition enhances cardiovascular responses to chemoreflex activation in awake rats

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Abstract

Several studies in the literature suggest that low-protein intake is associated with increases in sympathetic efferent activity and cardiovascular disease. Among the possible mechanisms, changes in the neurotransmission of cardiovascular reflexes have been implicated. Therefore, the present study comprised the evaluation of chemoreflex responsiveness in rats subjected to a low-protein diet during the 35 days after weaning. As a result, we observed that malnourished rats presented higher levels of baseline mean arterial pressure and heart rate and exhibited a mild increase in the pressor response to chemoreflex activation. They also exhibited a massive bradycardic response to chemoreflex activation. Interestingly, bilateral ligature of the carotid body arteries further increased baseline mean arterial pressure and heart rate in malnourished animals. The data suggest severe autonomic imbalance and/or change in the central interplay between neural and cardiovascular mechanisms.

Keywords: Arterial pressure; Chemoreflex; Albumin; Sympathetic activity; Low-protein diet

Introduction

Cardiovascular diseases are the most frequent causes of morbidity and mortality in the world. In the last few decades, scientific research has yielded great advances in cardiovascular disease diagnosis and therapy, however the need to better understand the pathophysiological mechanisms of cardiovascular diseases still exists. Regulation of the cardiovascular system invariably involves neural and hormonal systems, such as the sympathetic nervous system (SNS) and renin–angiotensin system (RAS), which play central roles in cardiovascular regulation in both health and disease. SNS involvement in the pathogenesis of hypertension, coronary artery disease or heart failure has been well-established (Sinski et al., 2006). Several studies have shown that reduced protein intake leads to changes in cardiovascular homeostasis, which affects peripheral vascular resistance, renin secretion, renal hemodynamics (reducing the renal blood flow and glomerular filtration rate) and central neurotransmission of cardiovascular reflexes pathways (Barker et al., 1990, 1993; Benabe et al., 1993a,b; Benabe and Martinez-Maldonado, 1993, 1998; Langley-Evans et al., 1996, 2003; Plagemann et al., 2000; Woods and Raju, 2001). Indeed, studies performed in our laboratory have shown that animals submitted to our malnutrition model (reduction of 60% in the dietary protein) are characterized by increased levels of baseline mean arterial pressure and suggest increased sympathetic efferent activity directed to the heart relative to normal diet-fed rats (Oliveira et al., 2004; Tropia et al., 2001). These studies also

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suggest that malnutrition affects cardiovascular homeostasis resulting in hypertension in this experimental model. Similar results were observed in humans (Sawaya et al., 2003) suggesting that the animal malnutrition represents a reliable model to study cardiovascular alterations following malnutrition states in humans. Nevertheless, mechanisms involved in the development of hypertensive states in malnourished animals should be explored further. A growing number of studies have suggested that the enhancement of sympathetic efferent activity could represent a risk factor for cardiovascular disease (Hawkins et al., 2000; Irigoyen et al., 2005; Young et al., 1985). We hypothesize that autonomic imbalance due to the low-protein diet could affect the sympathetic output to the heart and vessels and possibly contribute to the development of hypertension.

The chemoreflex is one of the most important cardiovascular reflexes involved in the maintenance of cardiovascular homeostasis. Activation of the chemoreflex response by cytotoxic or hypoxic hypoxia activates sympathoexcitatory and a parasympathoexcitatory efferent pathways resulting in a pressor and bradycardic responses, respectively (Barros et al., 2002). The activation of the chemoreflex response depends on the sensory mechanisms of glomic cells (Franchini and Krieger, 1992; Gonzalez et al., 1995; Marshall, 1994). Therefore, potassium cyanide (KCN) is an excellent tool to assess the chemoreflex response. The chemoreflex neural pathways are involved in the generation of hypertension, since chronic chemoreflex activation and increase in sympathetic efferent activity could lead to a sustained rise in mean arterial pressure (Fletcher, 2000, 2001; Fletcher et al., 2002; Tahawi et al., 2001).

The present study aimed to evaluate the chemoreflex response in malnourished animals to address whether this nutritional condition contributes to disruptions in the autonomic control of the cardiovascular system driven by the chemoreflex pathway.

Materials and methods

Animals

Male Fischer rats (180–210 g) from the Experimental Nutrition Laboratory of the Nutrition School were used in this study. The animals were kept in individual cages and fed with regular or low-protein diet and filtered water *ad libitum*. They were maintained in a climate controlled area (24 °C) on a 12-hour dark–light cycle of 12 h. All the experimental procedures are in accordance with the *Brazilian Council for Animal Experimentation* (COBEA).

Diets

To induce malnutrition, rats were fed with a normal or low-protein content diet manufactured at the Experimental Nutrition Laboratory of the Nutrition School. The regular protein diet contained 15% protein while the low-protein diet contained 6% protein. The diets were isocaloric (422 kcal/100 g of diet) and the salts and vitamins were at similar concentrations in both diets.

Malnutrition protocol

Two female per male Fischer rats (four months old) were maintained in plastic cages (47 × 33 × 15 cm) for mating. After 10 days, the animals were separated and kept in individual cages. During pregnancy and weaning periods, the females received regular rat chow and filtered water *ad libitum*. After the puppies were born, they were handled randomly, keeping eight puppies per mother, and the weaning period was set to 28 days. After the weaning period, the male rats were separated in individual cages and divided into four groups according to diet and ligature surgery: 1) control intact ($n=8$); 2) control ligated ($n=8$); 3) malnourished intact ($n=8$) and 4) malnourished ligated ($n=8$). The animals were maintained on these diet protocols for 35 days and carotid body artery ligation was conducted 1 day prior to the experiments. The experiments were conducted on the 36th day after weaning.

Blood measurements

Blood samples were collected and subsequent measurements of biochemical parameters (glucose, total proteins and albumin concentration) were performed before and after carotid body artery ligature. The blood samples were centrifuged at 4000 rpm for 10 min and the serum was kept in sterile centrifuge tubes and stored at −20 °C until the colorimetric analysis. For glucose measurements, the blood was centrifuged for 10 min at 2000 rpm. All measurements were performed using an automated system (Wiener Lab, Germany).

Catheterization of the femoral and artery vein

One day prior to the cardiovascular recordings, animals received polyethylene catheters into the femoral artery (for cardiovascular measurements) and vein (for systemic drug administration) under tribromoethanol (2.5%, Merck, Darmstadt, Germany) anesthesia. The catheters were tunneled through the subcutaneous and exteriorized on the back of the neck. The animals were maintained in individual cages in the experimental room until the next day to recover from anesthesia and adapt to the experimental room.

Ligature of the carotid body artery

We performed carotid body artery ligation to promote the degeneration of chemosensitive cells in the carotid body resulting in impairment of chemoreflex activation on two groups (normal protein diet fed and one low-protein diet fed) 1 day prior to experimentation, as described by Franchini and Krieger (1992).

Cardiovascular measurements

On the experimental day, the arterial catheter was connected to a pressure transducer MLT0699 (ADInstruments, Australia) and connected to a signal amplifier ETH-400 (CB Sciences Inc. USA). The pulsatile arterial pressure was recorded through a