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Heart dysfunction induced by choline-deficiency in adult rats: The protective role of L-carnitine

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ABSTRACT

Choline is a B vitamin co-factor and its deficiency seems to impair heart function. Carnitine, a chemical analog of choline, has been used as adjunct in the management of cardiac diseases. The study investigates the effects of choline deficiency on myocardial performance in adult rats and the possible modifications after carnitine administration. Wistar Albino rats ($n=24$), about 3 months old, were randomized into four groups fed with: (a) standard diet (control-CA), (b) choline deficient diet (CDD), (c) standard diet and carnitine in drinking water 0.15% w/v (CARN) and (d) choline deficient diet and carnitine (CDD+CARN). After four weeks of treatment, we assessed cardiac function under isometric conditions using the Langendorff preparations [Left Ventricular Developed Pressure (LVDP-mmHg), positive and negative first derivative of LVDP were evaluated], measured serum homocysteine and brain natriuretic peptide (BNP) levels and performed histopathology analyses. In the CDD group a compromised myocardium contractility compared to control ($P=0.01$), as assessed by LVDP, was noted along with a significantly impaired diastolic left ventricular function, as assessed by (−) dp/dt ($P=0.02$) that were prevented by carnitine. Systolic force, assessed by (+) dp/dt, showed no statistical difference between groups. A significant increase in serum BNP concentration was found in the CDD group ($P<0.004$) which was attenuated by carnitine ($P<0.05$), whereas homocysteine presented contradictory results (higher in the CDD+CARN group). Heart histopathology revealed a lymphocytic infiltration of myocardium and valves in the CDD group that was reduced by carnitine. In conclusion, choline deficiency in adult rats impairs heart performance; carnitine acts against these changes.

1. Introduction

Choline is a B vitamin co-factor and, although it is not by strict definition a vitamin, it is considered as an essential nutrient (Zeisel and Blusztajn, 1994). It is almost always found in the form of phosphatidylcholine or sphingomyelin in most foods (Zeisel et al., 2003). A choline deficient setting, due to inadequate dietary intake, is established in about two weeks (Pomfret et al., 1990; Zeisel et al., 1989,1991). Although the de novo biosynthesis of phosphatidylcholine, catalyzed by the enzyme phosphatidylethanolamine-N-methyltransferase (PEMT) found mainly in the liver, could probably compensate for the lack of dietary choline, in the cardiac tissue PEMT is considered quantitatively insignificant (Cui and Vance, 1996).

Choline seems to have, among other traits, a major physiological role in the development and function of the cardiovascular system in rodents and choline deficiency has been associated with significant cardiovascular morbidity or even mortality (Kesten et al., 1945; Newberne and Salmon, 1963; Repetto et al., 2010; Wilgram et al., 1954; Wilgram, 1957; Williams, 1960). Furthermore, choline is precursor of acetylcholine, which is a basic neurotransmitter of the autonomous nervous system that regulates chronotropic and dromotropic responses of the heart. However, most of the data regard weanling and young rats (Kesten et al., 1945; Wilgram et al., 1954, Wilgram, 1957; Repetto et al., 2010) and little is known about the effects observed in older rodents.

Carnitine, a tertiary amino-acid, is considered as a chemical analog of choline (Pieklik and Guynn, 1975). It is synthesized in liver and kidneys and is mainly stored in the skeletal and cardiac muscle (Dayanand et al., 2011). L-carnitine, the biologically active

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enantiomer, mediates the transport of long-chain fatty acids into the mitochondrial matrix for beta-oxidation and has proved to possess anti-oxidant and anti-inflammatory activity (Calò et al., 2006; Gómez-Amores et al., 2006; Mingorance et al., 2011; Silvério et al., 2011), in contrast to a choline deficient state that is associated with increased oxidative stress (Repetto et al., 2010). Due to its pharmacologic properties, carnitine has been recognized as a nutritional supplement in cardiovascular disease (Flanagan et al., 2010) and it is currently used as an adjunctive therapy in various heart conditions with promising results (Dayanand et al., 2011; Krim et al., 2013).

Carnitine is metabolized much more rapidly in choline-deficient rats than in normal rats (Mehlman et al., 1978; Tsai et al., 1975). In addition, choline can serve as methyl donor for the synthesis of carnitine from lysine and methionine (Griffith, 1987) and choline deficiency has been accompanied by carnitine deficiency (Corredor et al., 1967; Dodson and Sachan, 1996; Sheard and Krasin, 1994). This fact could further impair myocardial function (Zaugg et al., 2003) since cardiac muscle utilizes fatty acids as its primary energy source (Dayanand et al., 2011).

The present study was designed (a) to investigate the short-term effects of dietary choline deprivation on heart function and histology of healthy adult rats and (b) to evaluate the effects of carnitine administration on the eventual changes of the aforementioned parameters in a choline deficient state.

2. Materials and methods

2.1. Animals and diets

Male Wistar Albino rats ($n=24$), three months old (350+/−30 g body weight), purchased from the Greek National Center of Scientific Research 'Democritos', were used. After seven days of acclimatization at constant environmental conditions (room temperature 25+/−1 °C, humidity 45% and light/dark cycle 12/12 h), the rats were randomly assigned into 4 groups according to the following dietary pattern: (a) rats receiving standard diet (control-CA), (b) rats receiving standard diet and carnitine in drinking water 0.15% w/v, (c) choline-deprived rats receiving choline deficient diet (CDD) and (d) choline-deprived rats receiving choline deficient diet and carnitine in drinking water 0.15% w/v. The number of animals in each group was six. They were housed separately in stainless steel cages, while food and water were provided *at libitum*. Diets were purchased from AnaLab Ltd., Athens, Greece and L-carnitine was obtained by Vianex SA, Athens, Greece. The mean daily dose of L-carnitine used was 200 mg/kg body weight. The analytical composition (g/kg) of the choline deficient diet was: sugar 413, starch 110, dextrine 110, hydrogenated vegetable oil 100, pea protein 90, soya protein isolate 60, corn oil 50, mineral mix 35, vitamin mix 10, cellulose 10, vitamin free casein 10, L-cystine 2. The standard diet was enriched by choline (1.1 g/kg) at the expense of sucrose. The dietary intervention was imposed for four weeks in order to explore the early effects of dietary choline deprivation in the heart function of adult rats. All animal procedures were carried out in accordance with EEC-86/609 regulations under the authority of the relevant project license obtained from the Prefecture of Athens and were approved by the Institutional Animal Care and Use Committee of the University of Athens for Medical Sciences. The number of rats and the suffering were kept to a minimum as possible.

2.2. Isolated heart preparation

A non-working isolated rat heart preparation was perfused at a constant flow according to the Langendorff technique. An intraventricular balloon allowed measurement of left ventricular pressure under isovolumic conditions. Left ventricular balloon volume was adjusted to produce an average initial left ventricular end-diastolic pressure of 6–8 mmHg in all groups and was held constant thereafter throughout the experiment. Mean coronary perfusion pressure was adjusted between 67 and 72 mmHg in all experiments during the first 5 min of stabilization and kept constant thereafter. This resulted in mean coronary flow of 15.5 ml/min. No difference in coronary flow was found between groups. Intraventricular balloon was made from a flexible balloon catheter and latex membrane (Pantos et al., 2003,2007,2009). Since the balloon was not compressible, left ventricular contraction was isovolumic. As intraventricular volume was maintained at a constant value, diastolic fiber length, which represented preload, did not change. Thus, the left ventricular peak systolic pressure and the left ventricular developed pressure (LVDP), defined as the difference between left ventricular peak systolic pressure and left ventricular end-diastolic pressure, represented indexes of systolic function obtained under isometric conditions.

Rats were anaesthetized with ketamine HCl (100 mg/kg body weight) and heparin 1000 IU was given intravenously before thoracotomy. Ketamine was chosen, because it minimally affects contractile properties of the myocardium (Pantos et al., 2003,2007,2009). The hearts were rapidly excised, placed in ice-cold Krebs-Henseleit buffer (composition in mmol/L: sodium chloride 118, potassium chloride 4.7, potassium phosphate monobasic 1.2, magnesium sulfate 1.2, calcium chloride 1.4, sodium bicarbonate 25, and glucose 11) and mounted on the aortic cannula of the Langendorff perfusion system. Perfusion with oxygenated (95% O_2/5% CO_2) Krebs-Henseleit buffer was established within 60 s after thoracotomy. The perfusion apparatus was heated to ensure a temperature of 37 °C throughout the course of the experiment. Sinus node was removed after excision of the right atrium. All hearts were paced by epicardial placement of a platinum lead in the intraventricular septum at the base of the heart. Voltage and pulse duration of the pacemaker were adjusted at 9 V and 0.5 ms respectively, while the heart rate was set at 330 beats per min. This value of heart rate is within normal limits for rat species (Van Zutphen et al., 2001). The water filled balloon, connected to a pressure transducer and coupled to a Gould RS 3400 recorder was advanced into the left ventricle through an incision in the left atrium. Pressure signal was transferred to computer using data analysis software (IOX, Emka Technologies) which allowed continuous monitoring and recording.

Left ventricular function was assessed by recording the left ventricular developed pressure (LVDP-mmHg) and the positive and negative first derivatives of LVDP [(+) dp/dt and (−) dp/dt]. (+) dp/dt and (−) dp/dt (mmHg/s) are the sensitive indices of contractile function with respect to the rate of increase and rate of decrease of intraventricular pressure respectively. All preparations were perfused for 30 min and measurements were performed at the end of this period. All preparations included in this study were stable for at least the last 10 min of the perfusion period. LVDP as well as (+) dp/dt and (−) dp/dt values were recorded as mean values over a period of 30 s.

2.3. Histopathologic analysis

Following the evaluation of the mechanical heart function, the hearts were fixed after incubation in 10% formalin solution and then embedded in paraffin. Six sequential tissue sections, 4 mm in thickness, were taken from each heart, at a distance approximately of 2 mm from each other. Histological evaluation of the hearts was performed using Eosin-Hematoxylin and Masson stains. Randomly selected fields were examined under light microscopy. Myocardial inflammation, valvular inflammation, cardiac interstitial fibrosis,