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Supplementation with n-3 polyunsaturated fatty acids to lipopolysaccharide-induced rats improved inflammation and functional properties of renal Na,K-ATPase

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ARTICLE INFO

Article history:
Received 6 September 2012
Revised 5 June 2013
Accepted 13 June 2013

Keywords:
Sodium pump
Fish oil
Kidney
Rat
Inflammation

ABSTRACT

Measurements of enzyme kinetics of renal Na,K-ATPase were used for characterization of ATP- and Na^+-binding sites in rats that were subjected to 10 days of moderate inflammation that was induced by a single dose of *Escherichia coli* lipopolysaccharides (LPSs) at a dose of 1 mg kg^{-1} body weight. We hypothesized that LPSs might initiate a malfunction of renal Na,K-ATPase, which is a key enzyme involved in regulation of sodium homeostasis in the organism. We also investigated the potential effect that fish oil (FO) has in the prevention of Na,K-ATPase alterations by administering FO daily at a dose of 30 mg kg^{-1}. Alone, LPS elevated the level of C-reactive protein by more than 500% and free radicals by 36% in plasma, as indicated by an increased level of malondialdehyde. The Na,K-ATPase was slightly altered in the vicinity of the ATP-binding site as suggested by the 9% increase of the concentration of ATP necessary for half-maximal activation of the enzyme, thus indicating a deteriorated binding of ATP as a consequence of inflammation. Daily supplementation of FO partly attenuated LPS-induced injury, as observed by a significant decrease in the plasma levels of C-reactive protein and free radicals, hence maintaining the activity of renal Na,K-ATPase to the level of healthy control animals. In conclusion, our findings showed that FO prevented an excessive malondialdehyde production in LPS-treated animals and stabilized renal Na,K-ATPase.

1. Introduction

Inflammation induced by bacterial infection has been shown to disturb the maintenance of intracellular sodium homeostasis by Na,K-ATPase in various organs such as the pulmonary airway system [1–3], liver [4,5], heart [6], aorta [7], central nervous system [8,9], and kidney [10–12]. This enzyme plays a crucial role in cell homeostasis because it maintains Na^+ and K^+ gradients between the intracellular and extracellular milieu, which are necessary for maintenance of the cell

Abbreviations: C, control group; DHAs, docosahexaenoic acid; FO, fish oil; HR, heart rate; K_m, concentration of ATP necessary for half-maximal activation of the enzyme; LPS, lipopolysaccharides; MDA, malondialdehyde; n-3 PUFA, polyunsaturated fatty acid; NaCl, sodium chloride; ROS, reactive oxygen species; SBP, systolic blood pressure; V_{max}, maximum velocity.

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http://dx.doi.org/10.1016/j.nutres.2013.06.001

volume. The most important role in the regulation of sodium homeostasis in the whole organism is ascribed to the tubular Na,K-ATPase in renal tissue. The kidney, along with the pulmonary airway system and central nervous system, belongs to organs most susceptible to inflammation-induced complications. In the case of sepsis-associated acute renal failure, decreased expression of Na,K-ATPase was documented in the renal tissue of mice [11]. Despite intensive studies of Na,K-ATPase activity in various organs during bacterial infection, there is a lack of information about the molecular principles of Na,K-ATPase injury during inflammation. Omega-3 polyunsaturated fatty acids (n-3 PUFAs) were found to exert anti-inflammatory effects in various inflammatory diseases [13]. The modulation of inflammation by consuming bioactive food compounds such as n-3 PUFAs was shown to be a powerful tool to promote good health [14]. Furthermore, n-3 PUFAs are beneficial in the treatment of cardiovascular disease [15], immunoglobulin A nephropathy [16], and cyclosporine nephrotoxicity [17]. Supplementation studies that provided relatively high doses of n-3 PUFA have reported 30% to 55% decreases in the production of reactive oxygen species (ROS) by stimulated human neutrophils [18–20]. Previously, it was shown that an increased level of ROS induced the deterioration of the Na,K-ATPase function, and the negative roles of singlet oxygen [21,22], hydroxyl radical [23], hydrogen peroxide [24], alkoxyl radical [25], and superoxide radicals [26] have been well documented. Based on these observations, we hypothesized that supplementation of n-3 PUFA to rats that were subjected to LPS-induced inflammation might decrease the level of ROS in the organism and thus protect Na,K-ATPase activity. In an attempt to characterize the ATP- and Na^+-binding properties of renal Na,K-ATPase during LPS-induced inflammation and its treatment with fish oil (FO), the present study was designed to investigate the kinetic properties of the enzyme in male Wistar rats.

2. Methods and materials

2.1. Animal model

Experiments were performed on normotensive 3-month-old male Wistar rats that weighed approximately 220 to 240 g. Before the experiments, the rats were acclimatized and housed in temperature- and light-controlled conditions (23°C ± 1°C, 12-h light/12-h dark cycle). All rats were allowed free access to food and drinking water. The rats of all groups were given a standard rodent diet containing 10% kilojoules as fat (Table 1). At the beginning of the experiments, the rats were divided into 4 groups. The first (n = 12) group served as the control (C group). The second group (n = 12) of healthy Wistar rats was treated daily by an oral supplementation of FO in the amount of 30 mg kg^{-1} d^{-1} during a 10-day period (FO group). As the source of n-3 PUFA, the commercially available nutritional supplement by MaxiCor (Farmax, Hradec Králové, Czech Republic), which contained 57% eicosapentaenoic acid and 43% docosahexaenoic acid (DHA), was used. The third group (n = 11) of animals was vaccinated intraperitoneally with a single dose of *Escherichia coli* lipopolysaccharides (LPSs) in a dose of 1 mg kg^{-1} of body weight (LPS group). The last group (n = 11) was vaccinated like the third group, but from the day of the LPS injection, the rats were treated with FO for 10 days (LPS + FO group) in the same manner as the second group. Lipopolysaccharides [*E coli* serotype 055:B5, Sigma Chemicals (CHEMIE Gmbh Stenheim, Germany)] were dissolved in sterile 0.9% NaCl solution. Groups 1 and 2 were injected with the same amount of 0.9% sterile NaCl solution. At the end of the experiment, the anesthetized rats were euthanized by excision of the heart after intraperitoneal injection of thiopental at a dose of 65 mg kg^{-1}. The excised kidneys were immediately frozen in liquid nitrogen and stored for further investigation of Na,K-ATPase properties.

Table 1 – Diet components and total energy

Total energy (kJ/kg)	13 000
N-substances (g/kg)	240
Tassel (g/kg)	37.2
Fat (g/kg)	34.7
Calcium (g/kg)	13.2
Phosphorus (g/kg)	8.7
Sodium (g/kg)	1.8
Vitamin A (IU)	332 000
D3 cholecalciferol (vitamin D; IU)	2500
α-Tocopherol (vitamin E; mg/kg)	107.9
Copper sulfate pentahydrate (mg/kg)	22.6
Sodium selenite (mg/kg)	0.34

Standard rat chow (ST-1, 10.1% kJ from fat) was provided to all animals ad libitum. The groups of rats with FO supplementation were treated orally every day by nutritional supplement containing FO in an amount of 30 mg kg^{-1} d^{-1} during a 10-day lasting period. As a source of n-3 PUFA, the commercially available nutritional supplement MaxiCor (Farmax) containing 57% of eicosapentaenoic acid and 43% of DHAs was used.

All experiments were in accordance with the Veterinary Council of the Slovak Republic (Decree No. 289, part 139, July 9, 2003), and they conformed to the Principles of Laboratory Animal Care (National Institutes of Health publication 83-25, revised 1985).

2.2. Basic characteristics of experimental animals

Using the noninvasive method of tail-cuff plethysmography, systolic blood pressure (SBP) was measured at the beginning and the end of the experiment. Body weights of the animals were also measured at the beginning and end of the experiment, and the kidney weight and relative kidney weight were analyzed at the end of experiments only.

The inflammatory response was documented by analyzing the plasma levels of C-reactive protein (CRP). To determine CRP concentrations in the plasma, a commercial rat enzyme-linked immunosorbent assay kit from Immunology Consultant Laboratories, Inc (Portland, OR, USA), was used. The malondialdehyde (MDA) level, an index of lipid peroxidation, was determined by the double-heating method of Draper and Hadley [27]. The spectrophotometric measurement of the color produced during the reaction of MDA with thiobarbituric acid.

2.3. Preparation of plasmalemmal fraction for kinetic measurements

The plasmalemmal membrane fraction from the rat kidney was isolated according to the method described by Jorgensen