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Full Length Research Paper

The effect of L-carnitine on carbonic anhydrase level in rats exposed to exhaustive exercise and hypothermic stress

Elif Şıktar

Atatürk University, College of Physical Education and Sports, TR-25240-Erzurum, Turkey. E-mail: erelceydanur@hotmail.com. Tel: +90 442 2312234. Fax: +90 442 2360985.

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L-Carnitine is a quaternary ammonium compound biosynthesized from the amino acids lysine and methionine. It plays an important regulatory role in the mitochondria and is required for the transport of fatty acids from the cytosol into the mitochondria during the breakdown of lipids or fats for the generation of metabolic energy. The functions of L-carnitine in skeletal muscle are critical to sustaining normal bioenergetics during exercise. Carbonic anhydrase (CA; carbonate hydrolyase, EC 4.2.1.1) is a well-characterized pH regulatory enzyme in most tissues including erythrocytes and catalyzes reversible hydration of CO_2 to HCO_3^- and H^+. The only known physiological function of the CA isozymes is to facilitate the interconversion of CO_2 and HCO_3^-, Therefore they play key roles in diverse processes, such as physiological pH control and gas balance, calcification, and photosynthesis. In the present study, the effect of L-carnitine on carbonic anhydrase levels in rats exposed to hypothermic stress was investigated. For this purposes, 24 healthy Spraque Dawley male rats were divided into four groups: the first group made exhaustive swimming exercises at the temperature of 18°C; to the second group L-carnitine was given and exhaustive swimming exercises made at the temperature of 18°C; to the third group (sedentary group) only L-carnitine was given; and the last group (sedentary group) served as control. The results obtained from the present study demonstrated that the biggest inhibition was observed in the group that was given L-carnitine and made exhaustive swimming exercises at the temperature of 18°C. There were differences between groups 1 and 2, 3 and 4 ($p<0.05$).

Key words: L-Carnitine, hypothermic stress, carbonic anhydrase, enzyme, exercise.

INTRODUCTION

L-Carnitine plays important physiological roles shuttling the long-chain fatty acids across the inner mitochondrial membrane for β-oxidation and ATP production in peripheral tissues. It translocates acetyl-Co-A into cytoplasm during acetyl-L-carnitine transport out of mito-chondria. Despite the low level of β-oxidation in brain, L-carnitine is actively transported through the blood-brain barrier and accumulates in neural cells (Shug et al., 1982; Mroczkowska et al., 1997; Gülçin, 2006). As hypothesized (Shug et al., 1982; Nalecz and Nalecz, 1996), a major modulatory role for L-carnitine in neural function may be played through L-carnitine-mediated transfer of acetyl groups for acetylcholine synthesis, as well as by influencing signal transduction pathways and gene expression (Binienda and Ali, 2001; Gülçin, 2006).

L-Carnitine is derived from both dietary sources (75%) and endogenous biosynthesis (25%) in humans. It plays an essential role in human intermediary metabolism (Bremer, 1983; De Vivo and Tein, 1990). Furthermore, L-carnitine is an important cofactor of peroxisomal oxidation especially of very long-chain fatty acids due to the localisation of a carnitine acetyltransferase, matrix and cytosol-facing carnitine acyltransferases and a carnitine-acyl-carnitine translocase in peroxisomes (Ramsay, 1999; Gülçin, 2006). It plays a major role, as a cofactor, in the transportation of free faty acid (FFA) from cytosol to the mitochondria. FFA degrades to acyl-CoA by β-oxidation and these substances enter the tricarboxylic acid (TCA) cycle. A large amount of oxygen is consumed in this reaction and ATP is synthesized in the steps of the electron transport chain and oxidative phosphorylation. Oxygen is reduced to H_2O at the end of the TCA cycle

and the oxygen concentration decreases, thus ROS (reactive oxygen species) formation is reduced (Mayes, 2000). It was postulated that when L-carnitine is an endogenic help; the level of endogen carnitine changes with intense exercise and additional carnitine supports the differences that will be in the metabolism (Dhalla et al., 1991; Sahlin, 1990; Furat et al., 2006). Executive exercise may lead to reduction levels of L-carnitine (Janssens, 1998). Many researchers declared that L-carnitine supplement has beneficial effects on exercise performance and thus it increases fat oxidation during prolonged exercise, preserves glycogen stores and delays the onset of fatigue (Volek et al., 2001; Stephens et al., 2007). Greig et al. (1987) claimed that in researches they carried out with various different exercise, taking L-carnitine before exercise or increasing of acute carnitine has no effect on performance

Carbonic anhydrase (CA; Carbonate hydrolyase, EC 4.2.1.1) is a ubiquitous enzyme that catalyzes the reversible hydration/dehydration reactions of carbon dioxide and is involved in the biomineralization process. In the biomineralization process, the mineral structures involved are mainly calcium carbonate and calcium phosphate crystals, in invertebrates and vertebrates, respectively. This enzyme is a multi-functional enzyme that catalyzes the hydration/dehydration of carbon dioxide. The molecular characteristics of the CA across the plant and animal kingdoms are similar (Kim et al., 1983).

CA catalyzes the reversible hydration of CO_2 to HCO_3^- and H^+. In the red blood cell, this enzyme is necessary to facilitate the transport of carbon dioxide out of the body (Beydemir and Gülçin, 2004).

$$CO_2 + H_2O \rightleftarrows H_2CO_3^- \rightleftarrows H^+ + HCO_3^-$$

Many natural and synthetic substances can affect the living metabolism by altering enzyme activities and affecting metabolic pathways at low concentrations (Beydemir et al., 2002; Çoban et al., 2007). The inhibition of the carbonic anhydrase (CA, EC 4.2.1.1) isoforms I–XIV with simple, inorganic anions has been investigated in detail (Innocenti et al., 2009). For instance, melatonin and dantrolene which are used in medical treatment have been shown to inhibit erythrocyte HCA-I and HCA-II activities (Gülçin et al., 2004; Beydemir and Gülçin, 2004).

In view of these observations, the aim of this study was to investigate the effects of L-carnitine on carbonic anhydrase level in rats exposed to exhaustive swimming exercise and hypothermic stress.

MATERIALS AND METHODS

Chemicals

L-Carnitine was obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). All other chemicals used were analytical grade and obtained from either Sigma-Aldrich or Merck.

Animals and groups

In this study, 24 healthy Spraque Dawley male rats, weighing 250–300 g, 4–6 months of age were provided from Fırat University Experimental Animal Research Center (FUDDAM). The study was carried out in Atatürk University Research Center of Experiment Animals and the study was approved by the Ethical Committee of the Atatürk University (AUHADYEK, Ethical Committee Report No: 2008-51). All surgical procedures and protocols used here were in accordance with Guidelines for Ethical Care of Atatürk University Research Center of Experiment Animals.

The rats were kept under special conditions and were sheltered in cages, each with 6 rats, at the room temperature (25°C), supplying with food (Bayramoğlu Yem Sanayi, Erzurum, Turkey) and water for 12–h day and night cyclus. The rats were divided into four equal groups. Group 1: these rats made exhaustive swimming exercises at the temperature of 18°C; group 2: the rats were given L-carnitine and made exhaustive swimming exercises at the temperature of 18°C; group 3: the rats were given L-carnitine; group 4: sedentary control group. In the study, the L-carnitine was given to the rats 1 to 1.5 h before the exercises in the doses of 100 mg/kg by intraperitoneal (I.P.) route.

Exercise protocol

Maximal intensely tired swimming exercises were applied to exercise and L-carnitine exercise groups in test group.

Adaptation training

For the rats to have adaptation, they were first made to have swimming exercise in a pool, 80 x 60 x 60 cm^3 for 5 min at 28°C during 5 days (this temperature is the most appropriate for rat metabolism). A resistance of 2200 V and a digital thermometer (GEMO, micro software and PID thermo controlled device) were used to warm up the pool. After swimming exercise, the rats were dried with towels, made to rest for 30 min at a warm place and taken to cages.

Training of maximal loading exhausted swimming exercise

Those in exercise group (n: 12) were made to swim at 18°C until they felt tired. Beginning uncoordinated actions (inability to float by minor extremity actions), remaining under water for 10 s without swimming were determined as tiredness criteria (Osorio, 2003).

Determination of temperatures

American Health Assembly (AHA) approved of normal body temperature as 36.5–37.2°C. The body temperature of rats is the same as those of humans. A naked person can keep body inner temperature fixed between 12.5 and 55°C in dry weather (Ünal, 2002). For the body to feel the heat depends on the temperature of the weather, moisture rate and wind rate. 26–30°C is the optimal temperature for performance in water sports (Brooks and Fahey, 1985).

In this study the temperature was determined as 10°C less than average temperature of 28°C as optimal temperature for performance; under 10°C, hypothermic (18°C); over 10°C, hyperthermic (38°C). In the present study to determine temperature values of water, under 16°C and over 38°C posed risk for rats. The rats made to swim at 14 and 39°C died and had severe compli-cations in 5-10 min (three out of six).

Drawing of blood and preparation of haemolysate

Venous blood was drawn from the V cava inferior into a sterile plastic syringe (10 mL) using a sterile needle. Half of the drawn blood (3 mL) was added to a plastic test tube containing 50 µL of EDTA (1:100) to be used for the carbonic anhydrase enzyme activity assay. Erythrocytes were isolated from fresh rat blood after exhaustive exercise and hypothermic stress. Immediately, the fresh blood was centrifuged at low-speed centrifugation (1500 rpm) for 15 min (HERMLE Z 323 K) by removal of plasma and buffy coat. The erythrocyte pellet was washed three times with cold 0.16 M KCl and the supernatant discarded. One volume of erythrocyte pellet was suspended in five volumes of ice water to give an erythrocyte haemolysate. CA activity was determined colorimetrically as described below (Rickli et al., 1964; Wilbur and Anderson, 1948; Hisar et al., 2005; ArasHisar et al., 2004).

Protein determination

Quantitative protein determination was achieved by absorbance measurements at 595 nm according to Bradford's method (1976), with bovine serum albumin as standard (Gülçin et al., 2005; Şişecioğlu et al., 2009; Köksal and Gülçin, 2008; Şentürk et al., 2008).

Hemoglobin estimation

The hemoglobin (Hb) concentration in hemolysate was determined by the cyanmethaemoglobin method. All studies were performed at +4°C (Beydemir et al., 2003; Gülçin et al., 2005).

Carbonic anhydrase enzyme activity determination

Carbonic anhydrase activity was assayed by following the hydration of CO_2 at room temperature according to the method described by Wilbur and Anderson (1976). CO_2-hydratase activity as an enzyme unit (EU) was calculated by using the equation (t_o-t_c/t_c) where t_o and t_c are the times for pH change of the nonenzymatic and the enzymatic reactions, respectively (Beydemir and Gülçin, 2004).

Statistical analysis

The experimental results were performed in triplicate. The data were recorded as mean ± standard deviation and analyzed by SPSS (version 11.5 for Windows 2000, SPSS Inc.). For determining the mean of two groups different from each other, the Mean-Whitney U test which is a non-parametric test ($P<0.05$) regarded as significant, and $P<0.001$, very significant is used.

RESULTS AND DISCUSSION

L-Carnitine is an antioxidant and prevents the accumulation of end products of lipidoxidation (Fabriello and Calabrese, 1988; Lowitt et al., 1995). It is synthesized from two essential amino acids, lysine and methionine. In addition, L-carnitine is transported to skeletal and cardiac muscles after its major production in liver and kidney, and it transports Acyl-CoA acids into the mitochondrial matrix for β-oxidation of free fatty acids (Brevetti and Perna, 1992; Visioli et al., 1992; Gülçin, 2006).

L-Carnitine alters nitric oxide synthase activity in fibroblasts depending on the peroxisomal status (Koeck and Kremser, 2003). It had an effect on the prevention of experimentally induced myringosclerosis in rats. Also, it diminished the occurrence of myringosclerosis in rats after myringotomy possibly by antioxidant activity and decreasing the formation of ROS (Akbaş et al., 2003). Moreower, Yasuia and co-workers demonstrated that acetyl-L-carnitine has antioxidant activity towards oxidative stress, and that the improvement in cognitive ability seen with acetyl-L-carnitine may occur through an amelioration of cellular dysfunction via an inhibition of the increase in lipid peroxidation observed in the brain tissue of untreated senescence-acceleration-prone mice (Yasuia et al., 2002). L-Carnitine prevents oxidative stress and regulates nitric oxide, the cellular respiration (Brown, 1999) and the activity of enzymes involved in defense against oxidative damage (Kremser et al., 1995). Also, it had a protective effect on the activities of mitochondrial enzyme succinate dehydrogenase as well as the activity of the antioxidant enzymes, catalase and superoxide dismutase against 3-NPA-induced neurotoxicity (Binienda and Ali, 2001). The antioxidant defense system is composed mainly of three enzymes: glutathione peroxidase, catalase, and superoxide dismutase. L-Carnitine, being an antioxidant, can protect these enzymes from further peroxidative damage. It is very effective in normalizing age-associated alterations; it can be implemented in the aged to minimize age-associated disorders where free radicals are the major cause (Kalaiselvi and Panneerselvam, 1998). In addition, recent studies have shown that acetyl-L-carnitine, one of the short-chain acyl esters, enhances learning capacity in aging animals (Ando et al., 2001), improves the symptoms of nerve-degenerative disorders such as Alzheimer's disease (Pettegrew et al., 2000) and attenuates the neurological damage seen following brain ischemia and reperfusion (Calvani et al., 1999).

Many endogenic mechanisms serve in thermoregulation responses (Reilly et al., 2006). However, the literature related to the effects of L-carnitine on the exercise done hypothermic and hyperthermic ambient (Jansens et al., 1998) indicated that heat production decreased in exercising pigeons after L-carnitine supplementation. Exercise intolerance L-carnitine palmitoyltransferase enzyme deficiency has been postulated to depend on low-carbohydrate-high-fat diet, exhaustive exercise, fasting, hypothermia and insomnia (Orngreen et al., 2003); and especially, it created skeletal muscle damage (Gentili et al., 2008).

Various carbonic anhydrase (CA) isozymes are found in many different tissues and are involved in a number of different physiological processes, including bone resorption, calcification, ion transport, acid–base transport, and a number of different metabolic processes. In mammals, CA is expressed in almost all tissues.

Proposed functions include oxygen transport between lungs, red blood cells and tissues, pH regulation, ion exchange in the kidney, and electrical activity in the retina

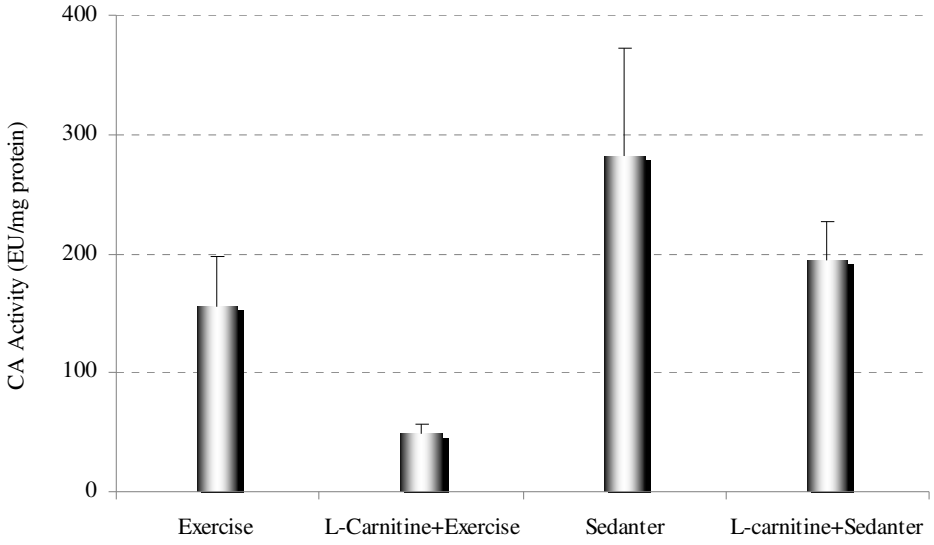

Figure 1. The inhibitor effect of L-carnitine on total carbonic anhydrase levels in rats exposed to exhaustive exercise and hypothermic stress. Exercise group made exhaustive swimming exercise at the temperature of 18°C; L-carnitine+Exercise group was given L-carnitine and made exhaustive swimming exercises made at the temperature of 18°C; L-carnitine+Sedenter group were administrated L-carnitine I.P.; and Sedenter (sedentary group) served as control.

and nervous system (Dogson, 1991; Chegwidden et al., 2000; Kummola et al., 2005). Another situation in which the role of CA has been extensively investigated is in autotrophic organisms, where CA is mostly related to the provision of bicarbonate for carbon fixation (Sultemeyer et al., 1993; Tsuzuki and Miyachi, 1989).

Many chemicals at relatively low dosages affect the metabolism of biota by altering normal enzyme activity, particularly inhibition of a specific enzyme (Hochster et al., 1972). The effects can be dramatic and systemic (Christensen et al., 1982). Indeed, CA isozymes are important enzymes in metabolism because they regulate pH in most tissues. CA inhibitors vary according to their affinity of binding to a particular CA isozyme, potency for inhibiting that isozyme and physicochemical properties, which can influence their tissue distribution and scope of activity (Beydemir et al., 2000)

In recent studies, it was shown that CA was potently inhibited by several members of sulphamate-containing drugs (Lloyd et al., 2005). Recently the effect of some drug, chemical or biocompounds had been extensively studied on carbonic anhydrase. For example, the effects of dantrolene sodium (Gülçin et al., 2004), melatonin (Beydemir and Gülçin, 2004; Hisar et al., 2005), α-tocopherol (ArasHisar et al., 2004), low molecular weight plasma inhibitors (Hisar et al., 2005), morphine (Çoban et al., 2007), ethanol (Çoban et al., 2008) and antioxidant phenols (Şentürk et al., 2009) were studied. Given the physiological importance of CA, the metabolic impact of medically important drugs should receive greater study. For example, in total hepatic CA activity was shown to diminish in the streptozotocin-induced diabetic rat (Thomsen and Charabi, 2000). Gluconeogenesis and ureagenesis were also associated with an increase in hepatic CA-V activity (Moynihan and Enis, 1990). There is convincing evidence for over expression of CA-IX and CA-XII in cancer (Türeci et al., 1998), acidifying the extracellular matrix, which is thought to promote growth of the tumour. Solid tumours are often hypoxic, and expression of CA-IX and CA-XII is upregulated under these conditions. CA present within the ciliary epithelium is associated with aqueous humour production and intraocular pressure. It is important for retina metabolism, acid–base homeostasis, and synaptic transmission (Feitl et al., 1991). In addition, hepatic pH disequilibrium was explained in terms of changes in CA activity. Furthermore, many drug side effects may be considered to result from CA isozyme inhibition. For example, respiratory acidosis is probably the cause of some side effects observed during acetazolamide therapy, such as fatigue, headache, altered taste sensations and distress (Thomsen and Charabi, 2000).

CA is present in a number of extrarenal tissues including eye, gastric mucosa, central nervous system and erythrocytes. Inhibition of CA in the ciliary processes of the eye decreases the rate of formation of aqueous humour and reduces intraocular pressure. Due to interference with CA activity in erythrocytes, CA inhibitors increase CO_2 levels in peripheral tissues and decrease amount of CO_2 in expired gas; therefore, acidosis may appear. On the other hand, large doses of CA inhibitors reduce gastric acid secretion (Jackson, 2001). CA inhibi-

tors are used for treatment of oedema, glaucoma or epilepsy. Acute mountain sickness is the most common and may appear at altitudes as low as 2000 m (Berkow, 1987). Acetazolamide is an effective prophylactic for acute mountain sickness. The mechanism for the beneficial effect of acetazolamide in acute mountain sickness is not clear, but it may be related to the induction of a metabolic acidosis (Jackson, 2001).

In this study, 24 healthy Spraque Dawley male rats (weighing 250–300 g and 4–6 months of age) were provided and divided into four groups: the first group made exhaustive swimming exercise at the temperature of 18°C; the second group was given L-carnitine and made exhaustive swimming exercises made at the temperature of 18°C; the third group were administrated L-carnitine I.P.; and the last group (sedentary group) served as control. As can be seen in Figure 1, the results obtained from the present study demonstrated that the biggest inhibition was observed in the group that was given L-carnitine and made exhaustive swimming exercises at the temperature of 18°C. There were differences between groups 1 and 2, 3 and 4 ($p<0.05$).In conclusion, the data presented in this study clearly showed that total carbonic anhydrase activity of rat blood was inhibited by L-carnitine in the rats exposed to exhaustive swimming exercises and hypothermic stress more than the rats exposed to only exhaustive swimming exercises.

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