Influence of Hyperthermic Environmental Temperature on Adenosine Deaminase Activity in Serum and Lymphoid Organs of Wistar Rats

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Abstract

Aim: To analyze the influence of the hyperthermic environmental temperature (35 ± 1°C) on the adenosine deaminase activity (ADA) in tissues rich with lymphoid cells in Wistar rats.

Material and Methods: The activity of ADA was examined in thymus, spleen, Payer’s patches, mesenteric lymph nodes and serum. The experimental animals (n = 11 male rats) were exposed on hyperthermic environment (35 ± 1°C) and relative air humidity of 20 - 30% within a period of 30 days. The control group of animals (n=10) were kept on room temperature (18 - 22°C).

Results: The body weight was decreased, spleen mass, and the thymus mass were reduced. Activity of ADA in the thymus was significantly increased from 10.58 ± 1.57 U/g in the control group of rats to 13.38 ± 2.7 U/g in rats exposed on 35 ± 1°C (p<0.01), and in the spleen it raised from 5.17 ± 3.29 E/g to 7.8 ± 1.97 U/g (p<0.05). The ADA activity in the other lymphoid organs and serum showed no statistically significant changes between the two groups of examined rats.

Conclusions: Influence of hyperthermic environment (35 ± 1°C, 30 days) provokes decrease in body weight, spleen and thymus relative mass, increase of the enzyme activity of ADA in thymus and spleen of rats, but there are no changes in the other lymphoid organs and tissues.

Introduction

Adenosine deaminase (ADA) is an enzyme which catalyses the irreversible catalytic deamination of adenosine and 2'-deoxyadenosine into inosine and 2'-deoxyinosine. This is a part of the purine graded way which enables adenosine detoxication from the organism. Adenosine has immunosuppressive effect, thus inhibiting the activation of T-lymphocytes, their proliferation and production of Il-2 [1]. In the early 1970-ties it was discovered that ADA deficiency in children was always transformed into combined immunodeficiency [2, 3] and since then, ADA has been a topic of many investigations. Immunodeficiency is result of lymphospecific toxicity of accumulated ADA substrates. Adenosine deaminase is an enzyme widely distributed in the tissues of the animals. In rats, the activity of ADA is the greatest in the spleen and thymus [4]. In man and other mammals, ADA exists...
in two isoenzyme forms: ADA1 and ADA2 [5]. ADA1 and ADA2 are coded from different gene loci [6, 7].

Ninety to one hundred percentage of the total intracellular ADA activity is due to ADA1, especially in cortical thymocytes of human thymus. Adenosines induce T cell apoptosis in vitro [3]. ADA1 prevents accumulation of toxic levels of 2’ - deoxyadenosine in lymphocytes and other immune effectors cells [8]. The importance of ADA1 for the development and function of the immune system is evident. ADA2 is an extracellular isoenzyme which determines the ADA activity in serum and plasma. ADA2 is used as a marker for HIV-1 seroconversion [9].

The elevated ambient environmental temperature, hyperthermic environment, is an essential ecological factor which provokes a series of qualitative and quantitative changes in the organism, the so called acclimation changes (in laboratory conditions), directed towards maintenance of the existence of the internal environment.

According to Eagan’s definition, the notion “acclimation” is used for: “Acclimation - the functional compensation over a certain period of days to weeks in response to a single environmental factor only, as in controlled experiments” [10]. According to Collins and Weiner [11], the hyperthermic environmental temperature is a stress on all levels of biological organisation which in homeothermic organisms induces a complex of biological functions, aiming at survival in the new conditions. A large number of experimental data pinpoint the influence of the elevated environmental temperature on the homeothermic organism. It is well known that the hyperthermic environmental temperature increases the body temperature, reduces the body weight and mass of certain organs and endocrine glands. Changes both in the enzyme system [12] and immune system [13-15] have also been noticed.

In light of the obvious connection of the enzyme ADA with the immune system, we have investigated the influence of the hyperthermia on the activity of this enzyme in organs rich with lymphoid cells.

The aim of this paper is to find out whether the hyperthermic environmental temperature affects the activity of the enzyme adenosine deaminase in lymphoid organs and tissues of rats.

Material and Methods

Experimental animals: Healthy male Wistar rats, at the age of 2 months, with body weight of 160 g (approximatively), were used.

Environmental temperature: The experiments were performed on two environmental temperatures. The control group was acclimated at room (laboratory) temperature (n=10), and the experimental one at the hyperthermic environmental temperature (n=11). The laboratory (room) temperature ranged between 18 and 22°C, whereas the hyperthermic environmental temperature - hot chamber (2*1.5*3m) was regulated on 35 ± 1°C, with relative air humidity of 20 to 30%. Both temperature environments were lighted up/illuminated for 12 hours per day (from 06:00 a.m. to 6:00 p.m.). Acclimation lasted for 30 days during which period the animals were given food and water ad libitum.

Processing of the animals in the experiment: Consequent to acclimation, the body weight of the animals was measured, after which they were sacrificed with ether anesthesia and aorta bleeding. Certain organs and tissues were removed, measured on a torsion balance, frozen in liquid nitrogen and stored on -20°C until further processing.

Examined parameters: The activity of the enzyme adenosine deaminase was examined in the following organs: thymus, spleen, Peyer’s patches, mesentheric lymph nodes and serum. The difference between the body weight of the animals and the relative mass of the relevant organs in both groups of animals, has been investigated. The body weight is expressed in grams (g) and the relative mass in milligrams per 100 grams body weight (mg/100 gr).

Method of measurement of the enzyme ADA activity: As soon as the tissues were unfrozen, they were homogenised in 5 ml phosphate buffer and centrifuged on 1800 rpm/15 min. The ADA activity was determined in 0.05 ml of supernatant in each tissue. The spectrophotometric method by Guisti from 1970, modified for our conditions, was used. The activity of the enzyme is expressed as units per gram tissue, wherein one unit is mmol per dissolved adenosine for one minute on 37°C [16].

Equipment used: Hot chamber (2*1.5*3m), container for liquid nitrogen, homogenizator (Polytron, Luzern), centrifuge model TJ-6 (Beckman, USA), torsion balance (Precision Balance, Federal Pacific Electric Co., USA), balance (Triple Beam Balance, Ohaus Scale Corporation Union, New Jersey, USA), water bath (Sutjeska, Zagreb), spectropho-tometer (Bio Merieux sa).
**Statistical processing**: The results were grouped and analyzed for arithmetic mean value, standard error and standard deviation, and the differences were analyzed with the Student’s t-test. The values for p<0.05 were considered to be statistically significant.

**Results**

The changes of the body weight and relative mass of the lymphoid organs and tissues under the influence of elevated ambient temperature (35 ± 1°C) in rats, are presented in Table 1.

<table>
<thead>
<tr>
<th>Ambient temperature (°C)</th>
<th>N</th>
<th>Body Weight (g)</th>
<th>Thymus (mg/100g)</th>
<th>Spleen (mg/100g)</th>
<th>Peyer’s Patches (mg/100g)</th>
<th>Mesenteric Lymph Nodes (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-22</td>
<td>10</td>
<td>175.4 ± 21.0</td>
<td>245.5 ± 30.8</td>
<td>375.9 ± 176.6</td>
<td>122.1 ± 19.1</td>
<td>97.2 ± 15.5</td>
</tr>
<tr>
<td>35 ± 1</td>
<td>11</td>
<td>154.7 ± 11.1</td>
<td>204.8 ± 29.9</td>
<td>237.6 ± 74.1</td>
<td>136.6 ± 29.8</td>
<td>92.2 ± 35.6</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Data are means ± standard deviation. P, values relate to differences from control; NS, not significant; N, number of rats.

It could be noticed that the elevated ambient temperature (35 ± 1°C) caused statistically significant decrease of the body weight from 175.35 ± 21 g in the control group to 154.69 ± 11.14 g in the experimental animals (p<0.015).

There was a statistical significance in the changes of the relative mass of both the spleen and the thymus. The spleen mass was reduced from 375.94 ± 176.56 mg/100 g to 237.59 ± 74.06 mg/100 g (p<0.03), and the thymus mass from 245.52 ± 51.55 mg/100 g to 204.84 ± 29.92 mg/100 g (p<0.04). Decrease of the relative mass of both Peyer’s patches and mesenteric lymph nodes was not statistically significant.

The differences in the activity of ADA enzyme in the lymphoid organs and tissues in rats exposed to different environmental temperatures are shown in Table 2.

ADA activity was significantly increased in the thymus from 10.58 ± 1.57 U/g in the control group of rats to 13.38 ± 2.7 U/g in rats exposed on 35 ± 1°C (p < 0.01), and in the spleen it raised from 5.17 ± 3.29 U/g to 7.8 ± 1.97 U/g (p < 0.04). The reduced ADA activity in the mesenteric lymph nodes and serum in the experimental group of rats was not statistically significant. ADA activity in Paer’s patches remained almost unchanged.

**Discussion**

The changes of the homoeothermic animals under the influence of the hyperthermic environmental temperature are polymorphic. The latest literature data have pointed out that the hyperthermic environmental temperature reduces the number of the cytotoxic activity of the spleen cells - natural killers (NK) in C3H/HeNCrj mice which are exposed on 35°C for 16 days. Body weight and relative mass of spleen remain unchanged [15]. In rabbits exposed on 33.5°C for 24 days, the capacity of polymorphonuclear cells for proliferation is decreased and at the same time, differentiation of the B lymphocytes in antibody secreting cells is inhibited [17]. Lymphocytes from the thymus and spleen exposed on 37°C in vitro have increased their proliferate activity [18]. Lymphocytes from the thymus and spleen exposed on 37°C in vitro have increased their proliferate activity [18]. In rats exposed on 34°C to 37°C for 24 hours, increased chemotaxis of the polymorphonuclear cells appears at the site [13]. It is evident that hyperthermic effects depend on the intensity, duration, and repetition and time exposition as well as on the type, age and state of the organisms.

The results obtained have shown that the elevated ambient temperature causes reduction of the body weight in healthy male rats as well as reduction of the mass of the lymphoid organs and tissues. These results coincide with the investigations of Dalal E [13] reporting that hypothermia brings about reduction of the mass of the thymus, increase of the level of corticosterone and it presents a stress for the organism. Body weight, thymus and spleen relative mass, decrease in BALB/C mice, exposed 15 days at 35°C [19].

During the early stages of hyperthermia, in the first 24-72 h, high corticosterone levels are measured in serum, whereas within long-duration of exposition (1 to 60 days), the level of corticosterone is significantly decreased and it slowly returned to its normal values from the 14th to the 60th day [12]. The influence of heat acclimation (1 to 48 h and 4 to 60 d at 35 ± 1 degrees C) on certain hepatic carbohydrate-related enzymes and substrates was investigated in rats. The time-dependent changes of duration of heat acclimation...
could be summarized in three phases: short-term heat exposure (1 to 24 h) with intensive glycogenolysis and gluconeogenesis to glucose; a period with temporary changes (24 h to 7 d) with tendency of normalization to control level, and prolonged heat acclimation (7 d to 60 d), which favours both direct and indirect glycogen synthesis [20].

It could be outlined that the elevated ambient temperature is a stress factor which, through the increased adrenal cortical activity, acts immuno-suppressively only in the first three days of the exposition to the elevated ambient temperature, after which acclimation follows. High concentrations of the corticosteroids decrease the activity of ADA, whereas the low concentrations increase it [4]. Recently, the time-dependent acclimatory changes of heart glycogen metabolism were investigated. Cardiac levels of key carbohydrate-related enzymes and substrates were studied in the function of the duration of short-term (STHA; 6, 12, 24 and 48 h) and long-term heat acclimation (LTHA; 7, 14, 21 and 30 days) to high environmental temperature (35 ± 1°C). The results obtained have showed that acclimation to moderate hyperthermic environment has caused significant changes in examined parameters which differ depending on duration to the exposure: intensive stress-induced glycogenolytic and glycolytic processes in the period of STHA and intensive energy sparing, manifested by Glk deposition in the period of LTHA [21].

Changes in the immune system of the rats influenced by the elevated ambient temperature were investigated. Male Wistar rats were divided, into 2 groups and housed at 20 ± 2 degrees C (n = 64, control group) and 35 ± 1 degrees C (n = 74, experimental group), during precise timing of 1, 4, 7, 14, 21, and 30 days. All the animals were given food and water ad libitum, and were lighted during 12 hours per day. IgG, IgG1, IgG2a, IgG2b and IgG2c subclasses were measured by radial immunodiffusion. The obtained results showed significant elevation in the level of IgG after 4 and 7 days (+32%), IgG2a after 7th (+88%), 14th and 21nd day (+110%), IgG2b after 14 days (+60%) at 35 ± 1 degrees C compared with the control group at 20 ± 2 degrees C. IgG1 level was not affected and IgG2c showed significant decrease after 21st day at 35 ± 1 degrees C. It was concluded that during the elevated ambient temperature the immune system is activated as one of the regulation mechanisms in homeostasis and survival of the population [22].

In the recent paper it was induced heat acclimation in 6 healthy men by 100 min of heat exposure for 9 days. Heat exposure consisted of (1) 10 min of immersion up to chest-level in water at 42°C and (2) 90 min of passive heating by a warm blanket to maintain tympanic temperature at 37.5°C. The climatic chamber was maintained at 40°C and a relative humidity of 50%. Blood samples were analyzed before and after heat acclimation for natural killer (NK) cell activity, counts of lymphocytes B and T, before and after heat acclimation for peripheral blood morphology, interleukin 6, tumour necrosis factor alpha, and cortisol. A Japanese version of the profile of mood states questionnaire was also administered before and after acclimation. The concentrations of white blood cells, lymphocytes B and T, cortisol, interleukin 6, tumour necrosis factor alpha and NK cell activity showed no significant differences between pre- and post-acclimation, but there was a significantly lower platelet count after acclimation and, with the profile of mood states questionnaire, there was a significant rise in anger after acclimation. It was concluded that heat acclimation by passive heating does not induce alterations in immune or endocrine responses [23].

Our investigations have shown that, in spite of the statistically significant decrease of the mass of the spleen and thymus in rats exposed on 30°C, the ADA activity in these organs was significantly increased. We assume that the level of corticosterone was still low on the 30th day of the exposition to hyperthermic environmental temperature and it stimulated the ADA activity. The ADA activity in serum, mesenteric lymph nodes and Paer’s patches was not statistically significant. We assume that the hyperthermia selectively destroy cells with lower ADA activity, while the resisting cells have higher ADA activity (thymus, spleen).

It remains under further examination to discover whether the high ambient temperature, stimulating the activity of the enzyme adenosine deaminase in thymus and spleen, stimulates the immune system of the male Wistar rats.

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References


