EFFECT OF SHORT TIME EXPOSURE OF RATS TO EXTREME LOW TEMPERATURE ON SOME PLASMA AND LIVER ENZYMES

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Received for publication October 10, 2005.

Abstract

The aim of the study was to search the influence of cryotherapy on liver enzyme activity in experimental rat model. The first group of rats was exposed 1 min daily to -90°C for 5 d, the second group was exposed 1 min daily to -90°C for 10 d and the control group was not exposed to low temperature. A statistically significant increase in the activity of glutamate dehydrogenase, sorbitol dehydrogenase, malate dehydrogenase, ornithine transcarbamoylase and arginase was observed in the plasma and liver. The obtained results indicate the influence of low temperature on liver metabolism.

Key words: rat, cryotherapy, liver enzymes, metabolism.

There is growing interest in using extreme low temperature in medical treatment. Cryotherapy makes the use of low temperature applied for a short time to stimulate physiological reaction of an organism to this condition (16). Whole-body cryotherapy results in analgesic, antiswelling, immune, hormone and circulatory system reactions (4). The metabolic effects of cryotherapy are not yet clear (17). One of the fundamental body functions is an thermoregulation within a defined temperature range. Thermal homeostasis is necessary for correct run of every metabolic process. During cryotherapy, thermoregulation is affected by an increase in heat production in the organism caused by an elevated intensity of metabolism (1). The aim of our study was to search the influence of cryotherapy on liver enzyme activity in experimental rat model.

Material and Methods

Studies were carried out on 18 adult (3 month-old) male Wistar FL rats (body weight about 320 g). The protocol of the studies was reviewed and approved by the Bioethical Committee of Medical University of Silesia in Katowice. Animals were supplied by the Experimental Animal Farm. The rats underwent two week environmental adaptation cycle. After this period the animals were randomized into 3 equal groups. Group I - rats exposed to -90°C for 5 d; group II - rats exposed to -90°C for 10 d and group III - control rats – without exposure to cryotherapy. All the groups were fed standard rat chow ad libitum.

The rats were put individually into cold chamber in wooden cages. Body weight of the animals was controlled before and after 5 and 10 d of the experiment. During ether anaesthesia of the animals blood was taken from the right ventricle of the heart and collected into EDTA-containing tubes. Then the animals were killed by the by spinal cord disruption. Ten per cent homogenates of the liver were prepared in 0.9% sodium chloride solution with the use of Potter-Elvehjem homogenizator. The homogenates were stored at –20°C for 36 h. After defrosting, the homogenates were treated by ultrasounds (ultrasonic desintegrator UD-11). Total protein, glucose, creatinine and urea concentrations were measured in the plasma. The activity of glutamate dehydrogenase (GDH), malate dehydrogenase (MDH), and sorbitol dehydrogenase (SDH) were determined in the plasma and liver homogenates, and the activity of ornithine transcarbamoylase (OTC) and arginase (ARG) was measured in liver homogenates.

Total protein, glucose, creatinine, and urea contents were measured with the use of standard diagnostic kits. The activity of GDH was measured with the use of spectrophotometric method by Schmidt (11). The activity of MDH was measured with the use of colorimetric method by Więckowski (4). The activity of SDH was measured with the use of spectrophotometric method by Gerlach (6). The activity of OTC was determined with the use of colorimetric method by Zak...
The activity of ARG was measured with the use of colorimetric method by Tamir et al. (13).

Statistical analysis was performed using STATISTICA 6.0 PL. The differences between groups were analysed using U-Mann-Whitney test. To be considered statistically significant, we required P<0.05 (confidence limits).

Results

A comparative assessment of plasma protein, glucose, creatinine, and urea concentrations in rats subjected to different conditions of cryotherapy are shown in Table 1. There were no statistically significant changes between the studied groups. However, there was statistically significant increase in the activity of plasma and liver GDH as compared to the control group (Figs 1 and 4). MDH activity was significantly increased in the liver of animals kept at low temperature comparing to the control (Fig. 5). There was also statistically significant increase in plasma and liver activity of SDH in comparison to the control group (Figs 2 and 3). Liver OTC and ARG activity showed statistically significant increase in groups exposed to low temperature as compared with the control (Figs 6 and 7).

There were no statistically significant changes in all the examined parameters between groups exposed for 5 d and 10 d to cryotherapy.

Table 1
Protein, glucose, creatinine and urea concentrations in the plasma

<table>
<thead>
<tr>
<th></th>
<th>-90/I</th>
<th>-90/II</th>
<th>control</th>
</tr>
</thead>
<tbody>
<tr>
<td>protein g/l</td>
<td>65.8±16.5</td>
<td>65.7±18.5</td>
<td>66.4±18.2</td>
</tr>
<tr>
<td>glucose mmol/l</td>
<td>5.90±1.25</td>
<td>5.33±0.96</td>
<td>4.52±1.02</td>
</tr>
<tr>
<td>creatinine mmol/umol</td>
<td>39.8±19.6</td>
<td>37.1±16.3</td>
<td>43.5±11.9</td>
</tr>
<tr>
<td>urea mmol/l</td>
<td>6.17±1.29</td>
<td>6.30±0.99</td>
<td>6.31±1.17</td>
</tr>
</tbody>
</table>

Fig. 1. Plasma GDH activity in rats exposed to -90°C compared to the control.

Fig. 2. Plasma SDH activity in rats exposed to -90°C compared to the control.

Fig. 3. Liver SDH activity in rats exposed to -90°C compared to the control.

Fig. 4. Liver GDH activity in rats exposed to -90°C compared to the control.
Discussion

A constant level of body temperature can only be preserved if the heat production by the organism is balanced by its heat loss, which is achieved by physiological mechanism of thermoregulation. One of the factors determining the intensity of metabolism and consequently the rate of heat production, is the temperature of surroundings. In biochemical thermoregulation the liver plays a crucial role. The temperature of the blood in the hepatic vein is higher than that in the hepatic artery, which points to active heat production in the liver. This process is intensified when the body is cooled during cryotherapy. A very important question is the influence of cryotherapy on individual metabolic pathways. In our study we focused on liver enzyme activity.

Important indicator enzyme involved in protein metabolism is GDH. On the basis of the performed experiment we observed increased activity in GDH in groups subjected to cryotherapy at -90°C. This increase was observed in the plasma as well as in the liver of examined animals.

GDH is situated in the mitochondrium. This is the regulatory enzyme which is under influence of an activator (ADP) and inhibitors (ATP, GTP, NADH). Intensification of heat production during cryotherapy is connected with some ATP degradation in thermogenesis processes. As the effect, we have more ADP. Conversion of α-amino groups of amino acids is catalyzed by GDH in the process called transdeamination (10). It is a cross reversible reaction both in catabolism and glutamate anabolism. Transdeamination leads to the occurrence of ammonia. This is a very toxic product even in the minimal concentration. The main mechanism of ammonia detoxication in the liver is production of urea in the urea cycle (2).

In our experiment an increase in the GDH activity was accompanied by the increase in liver OTC activity. OTC is the key enzyme of urea cycle. This enzyme, just as the GDH, is present in the liver mitochondrium and catalyses conversion of carbamoyl phosphate to ornithine (9).

Other important urea cycle enzyme, arginase, was also increased during the cryotherapy. The low temperature exert a great influence on metabolic pathways through changes in the activity of hormones. Concentration of amino acids in blood is the main factor adjusting conversion of nitrogen in the liver. Anabolic hormones lower the concentration of amino acid in blood through their stimulation of catching by skeleton muscles. As the result, liver amino acids uptake falls off. Then, catabolic hormones increase the supply of amino acid in the liver and utilization in the process of protein resynthesis, as well as they intensify the process of ureapoiesis. We supposed that intensification of protein catabolism during cryotherapy is connected with modification of hormonal regulation of this process (7). We have not observed any changes in serum urea and creatinine concentration in rats exposed to -90°C. There

Fig. 5. Liver MDH activity in rats exposed to -90°C compared to the control.

Fig. 6. Liver ARG activity in rats exposed to -90°C compared to the control.

Fig. 7. Liver OTC activity in rats exposed to -90°C compared to the control.
were no changes in serum protein concentration during the cryotherapy.

An increased MDH activity in the liver during the cryotherapy was observed. There were no statistically significant changes in serum MDH activity. Increase in liver MDH activity may indicate the enhanced transport of reducing equivalent by mitochondrial membrane in shuttle system (5). This transport gets through the couple of enzymes – malate dehydrogenase and aspartate dehydrogenase. Increased activity of tissue oxidation processes in low temperature condition leads to the increase in heat production at the cost of oxidative phosphorylation process. MDH takes part in supplying acetyl-CoA and NADPH for fatty acid biosynthesis and in supplying substrates for gluconeogenesis (12). A statistically significant increase in SDH activity during the cryotherapy was observed. Increased SDH activity was found in the plasma and liver of the examined animals. This is NAD dependent enzyme which catalyzes conversion of sorbitol to fructose. In the glycolysis process fructose is metabolized more rapidly than glucose. It is due to the fact that fructose passes over the reaction catalyzed by phosphofructokinase. Fructose intensifies fatty acid biosynthesis. It is supposed that at low temperature increased activity of sorbitol pathway makes possible glucose utilization in spite of saturate glycolyse pathways (3). SDH is the cytoplasm enzyme. Simultaneous increase in the plasma and liver SDH activity points at migration of this enzyme outside the hepatocytes. Concentration of glucose did not differ between animals subjected to the cryotherapy and the control group. Activation of glucose metabolism pathways in the liver assures constant glucose concentration in plasma in low temperature condition (8).

It is known that starvation, as well as physical effort, leads to energy deficiency and, as a result, to the increased activity of some enzymes. It is supposed that temperature -90°C causes increased activity of some enzymes to compensate energy deficiency and maintains the constant body temperature. There may be some different causes of the increased enzyme activity during cryotherapy. It may be increase in the concentration of enzymatic protein, it may be due to blockade of inhibitors of some enzymes in low temperature condition, or it may be the consequence of changes in condition of enzyme transport between the liver and plasma (15).

References