Lipid Peroxidation in Rat Brain is Increased by Simulated Weightlessness and Decreased by a Soy-Protein Diet

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Abstract. This study tested whether or not simulated weightlessness by tail-suspension increases the levels of lipid peroxidation products in rat brain. The brain tissues of rats on a soybean diet were also assayed for lipid peroxidation products to evaluate the possible role of soy-protein as a dietary anti-oxidant. Male Sprague-Dawley rats were used. Group 1 rats were fed standard Purina rat chow ad libidum and served as controls. Group 2 rats were fed a soybean diet containing 37% soy-protein and were not tail-suspended. Group 3 rats were fed standard Purina rat chow ad libidum and were tail-suspended to induce simulated weightlessness. After 2 wk, all of the rats were killed. Each whole brain was segmented into frontal cortex, cerebellum, and brain stem. After a specific weight of each segment was excised, the residual tissues were combined and used as a whole brain sample. The samples were analyzed for lipid peroxidation products by a chromogenic assay that reacts with malondialdehyde (MDA) and 4-hydroxyalkenals (4-HNE). The mean concentrations of lipid peroxidation products (MDA plus 4-HNE) in whole brain, frontal cortex, cerebellum, and brain stem of the control rats ranged from 16 to 18 µmol/g; the corresponding means ranged from 10 to 13 µmol/g in rats fed the soybean diet, and from 22 to 26 µmol/g in the tail-suspended rats. Thus, the mean levels of lipid peroxidation products in brain tissues were decreased in the rats fed the soy diet and were increased in the rats that were tail-suspended to simulate weightlessness, when compared to those of rats fed a regular diet. (received 10 October 2001; accepted 8 November 2001)

Keywords: lipid peroxidation, malondialdehyde, hydroxyalkenals, weightlessness, soy-protein diet

Introduction

Weightlessness in space is known to influence the physiological functions of the cardiovascular system, kidney, bone, muscle, and central nervous system [1-6]. An ideal animal model for studies of physiological functions under weightless conditions at sea level has not been established. However, tail-suspension of rats is a documented experimental model of microgravity that mimics certain physiological responses to weightlessness [7-11]. Using this procedure, we observed pathophysiological changes in the pancreas of rats that were tail-suspended for 2 wk [12,13]. Studies by others showed that cultured lung and pancreatic rudiments become differentiated during weightlessness [14].

The primary goal of the current study was to examine the effects of rat tail-suspension on lipid peroxidation products in brain tissues, as a marker of stress responses to simulated microgravity. Aldehydes such as malonaldehyde (MDA) and 4-hydroxy-alkenals (4-HNE) are products of the peroxidative degradation of polyunsaturated fatty acids and related esters [15-17]. Measurements of tissue levels of these products provide a convenient index of lipid peroxidation [18,19].

Since phytoestrogens reportedly have beneficial antioxidant effects [20-23], a secondary goal was to test the effect of dietary phytoestrogens on the levels of lipid peroxidation products in brain tissues in rats fed a soy-protein diet.
Materials and Methods

Male Sprague-Dawley rats (N = 40, initial body wt 200-225 g) were randomly assigned to three groups: control group (N = 10), soy diet group (N = 10), and tail-suspended group (N = 20). The protocols and procedures were in accord with the Guiding Principles of the Care and Use of Animals of the Council of the American Physiological Society and were approved by our Institutional Committee on Animal Care and Use.

The rats were kept at 30°C in a room with 12 hr light-dark cycle. The tail-suspended rats were able to move freely in their cages using their forelimbs. The control and tail-suspended groups were fed Purina rat chow, and the soy diet group was fed a diet containing 37% soy protein (Test Diet Co., Richmond, IN). During the 2-wk study period, the rats were monitored daily for food and water intake, as well as body wt.

After 14 days, the rats were fasted overnight, anesthetized with ketamine hydrochloride and acyl promazine, and killed by exsanguination. The entire brain was removed and immediately frozen in liquid nitrogen. Upon thawing, each brain was segmented into frontal cortex, cerebellum, and brain stem. After a specific weight of each segment was excised, the residual tissues were combined and used as the whole brain sample. Each sample was weighed, washed in ice-cold NaCl solution (9 g/L), minced, and homogenized in Tris-HCl buffer, pH 7.4 (tissue to buffer ratio, 1:10 w/v). The homogenate was centrifuged (10 min, 3000 x g, 4°C). The supernatant was diluted further (1:10, v/v) in Tris-HCl buffer and 200 µl aliquots were assayed for lipid peroxidation products, using the Bioxytech LPO-586 kit (R & D Systems, Minneapolis, MN).

The LPO-586 assay is based on reaction of a chromogenic reagent with malondialdehyde (MDA) and 4-hydroxyalkenals (4-HNE) at 45°C. One molecule of either MDA or 4-hydroxyalkenal reacts with 2 molecules of the reagent to yield a chromophore (maximum absorbance, 586 nm) that is stable for ≥ 1 hr at room temperature. The absorbance at 586 nm is a linear function of MDA or 4-HNE concentrations from 0 to 20 µM. The detection limit is 0.1 µM of MDA or 4-HNE. Each assay included a standard curve for MDA and/or 4-HNE. The levels of lipid peroxidation products in the brain tissue samples were expressed as µmol/g (wet wt).

Results were calculated as means ± SE. Statistical significance was determined by the unpaired t test (p < 0.05 was considered significant).

Results

In the tail-suspended group of rats, the intake of food and water was reduced during the initial 48 hr of suspension. After that interval, the food and water intakes of the control and the tail-suspended groups did not differ significantly. Food and water intakes of rats in the control and soy diet groups did not differ significantly throughout the study period.

Consistent with our previous report [24], mean body weights of the tail-suspended group were significantly reduced throughout the study period, compared to control and soy diet groups. Body weight gains did not differ significantly in the control and soy diet groups (data not shown).

Table 1. Lipid peroxide levels (mean ± SE, expressed as µmol MDA or 4-HNE/g, wet wt) in brain samples of the three groups of Sprague-Dawley rats

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (N = 10)</th>
<th>Group 2 (N = 10)</th>
<th>Group 3 (N = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet:</td>
<td>Purina rat chow;</td>
<td>Soy diet;</td>
<td>Purina rat chow;</td>
</tr>
<tr>
<td>Treatment:</td>
<td>none</td>
<td>none</td>
<td>tail-suspended</td>
</tr>
<tr>
<td>Lipid peroxide levels (µmol/g wet wt)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>18.03 ± 1.62</td>
<td>10.33* ± 1.07</td>
<td>21.56* ± 1.42</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>17.47 ± 2.17</td>
<td>13.20 ± 1.29</td>
<td>25.96* ± 1.40</td>
</tr>
<tr>
<td>Brain stem</td>
<td>16.55 ± 0.94</td>
<td>13.31 ± 1.67</td>
<td>22.73* ± 1.15</td>
</tr>
<tr>
<td>Whole brain</td>
<td>17.35 ± 0.93</td>
<td>12.25* ± 0.79</td>
<td>23.42* ± 0.79</td>
</tr>
</tbody>
</table>

* p < 0.05 vs corresponding value in controls (Group 1).
Levels of lipid peroxidation products (expressed as µmol of MDA or 4-HNE/g of tissue) in the frontal cortex, cerebellum, brain stem, and whole brain of rats in the 3 groups are listed in Table 1.

The mean levels of lipid peroxidation products in all brain samples were significantly higher (p < 0.05) in the tail-suspended group, compared to the controls. In the frontal cortex, cerebellum, brain stem, and whole brain of tail-suspended rats, the levels of lipid peroxidation products averaged, respectively, 19, 49, 37, and 35% higher than the controls.

The mean levels of lipid peroxidation products in the whole brain and frontal cortex samples were significantly lower (p < 0.05) in the soy diet group, compared to the control group. In the frontal cortex, cerebellum, brain stem, and whole brain of tail-suspended rats, the levels of lipid peroxidation products averaged, respectively, 43, 24, 20, and 29% lower than the controls.

Discussion

This study examined the effect of simulated microgravity on levels of lipid peroxidation products in rat brain tissues as a measure of stress response and also tested the effect of a soy diet in reducing the levels of lipid peroxidation products.

Our laboratory has investigated the effects of rat tail-suspension on some physiological functions, considering tail-suspension as a simulated model of microgravity [12,13,24-26]. To what extent the changes of function are the result of a centrally induced stress response has not been examined.

In the present study, as shown in Table 1, lipid peroxidation products increased significantly in brain tissues of tail-suspended rats, compared to those of controls, suggesting that tail-suspension induced a centrally activated cellular stress response. Bondarenko et al [27] studied the effects of chronic emotional stress on lipid peroxidation in the brain and other tissues of rats. The contents of lipid peroxidation products in brain, myocardium, and blood were higher in emotionally stressed rats, compared to controls. Furthermore, the increased levels of lipid peroxidation products correlated with the severity of pathological somatic manifestations.

These results have been corroborated in other forms of stress [28,29].

Guliaeva et al [30-33] investigated the influence of the duration of chronic emotionally painful stress on lipid peroxidation in the brains of rats. After 1 wk of such stress they reported inhibition of lipid peroxidation and activation of superoxide scavenging activity in rat brain. After 2 wk of stress, they found normalization of behavior and a low level of lipid peroxidation. After 3 wk of stress, hyperactivity was observed, with increased lipid peroxidation and decreased phospholipid content in rat brain. These results are in general agreement with the findings of the present investigation.

Levels of the chromogenic products of lipid peroxidation products were significantly elevated in whole brain homogenates of tail-suspended rats, compared to non-suspended controls. Furthermore, the lipid peroxidation products were consistently increased in homogenates of brain stem, cerebellum, and cerebral cortex of the tail-suspended rats, compared to the corresponding homogenates of non-suspended controls.

Arora et al [23] reported that isoflavones and their metabolites show antioxidant activities in a liposomal system. Isoflavonoids, a class of phytochemicals found in fruits, vegetables, and grains, have a restricted distribution in plant species, occurring almost exclusively in legumes. The isoflavones (eg, genistein and diadzein) of soybeans represent major dietary sources. Soy protein-containing diets have attracted attention because of their possible cancer-preventing properties [23]. Arora et al [23] showed that genistein, diadzein, and their glycosalated and methoxylated metabolites have antioxidant properties in liposomes and they studied the relevant structure-activity relationships.

Recently, Vedavanam et al [34] reported potential antidiabetic properties of an isoflavonoid-containing soybean extract, which reduced glucose-induced lipid peroxidation. Goldfarb [35] reviewed the antioxidant actions of isoflavonoids for prevention of exercise-induced muscle damage. Mizutani et al [36] showed that phytoestrogens attenuate oxidative DNA damage in vascular smooth muscle of spontaneously hypertensive rats. Chan and Yu [37] reported that an isoflavonoid, genistein,
inhibited UV irradiation-induced oxidative stress and apoptotic changes in human epidermal carcinoma cells. Haraguchi et al [38] found that certain isoflavones protect mitochondrial functions against oxidative stress.

Based on these reports, we studied the influence of a soy diet containing isoflavones on lipid peroxidation products in the brains of control, non-suspended rats. The results showed that the chromogenic products of lipid peroxidation (eg, MDA, 4-HNE), were significantly reduced in homogenates of whole brains or cerebral cortex of rats that received the soy diet. Further studies are needed to test whether or not diets with varied contents of soy isoflavones ameliorate the elevated levels of lipid peroxidation products in brains of tail-suspended rats.

In summary, tail-suspended rats had increased levels of lipid peroxidation products in brain tissues, compared to non-suspended controls; this suggests that simulated microgravity may cause stress-induced brain damage. Moreover, the rats fed a soy diet had decreased levels of chromogenic lipid peroxidation products in brain, compared to controls that were fed a standard diet of Purina rat chow.

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References


