EFFECT OF A SHORT TERM LATERAL ELECTRICAL SURFACE STIMULATION OF PARAVERTEBRAL MUSCLES ON BODY MASS AND MORPHOLOGY OF THE ADRENAL GLANDS IN RABBITS

JÓZEF SZAREK, IRENEUSZ M. KOWALSKI1, JOANNA WOJTKIEWICZ2, IZABELLA BABIŃSKA, WOJCIECH KIEBZAK3, MICHAŁ GESEK, AND MAGDALENA SZWEDA

Chair of Pathophysiology, Forensic Veterinary Medicine and Administration, Faculty of Veterinary Medicine, University of Warmia and Mazury, 10-719 Olsztyn, Poland
1Chair of Rehabilitation, Faculty of Medical Sciences, University of Warmia and Mazury, 11-015 Ameryka, Poland
2Department of Human Physiology, Faculty of Medical Sciences, University of Warmia and Mazury, 10-081 Olsztyn, Poland
3Department of Physiotherapy, Faculty of Health Science, University in Kielce, 25-734 Kielce, Poland
Szarek@uwm.edu.pl

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Abstract

The study was carried out on 10 male rabbits, New Zealand pure-bred, 3.5 months old, and weighing 2,000–2,200 g. The animals were divided into two equal groups: group 1 – subjected to short term lateral electrical surface stimulation (ST-LESS) for 2 h daily during 3 months, group 2 – control group without LESS stimulation but with other experimental parameters as in animals of the group 1 (SCOL-2 apparatus and preparation the skin to electrostimulation). Stimulation was performed with a Polish stimulator – SCOL-2, and clinical, macroscopic, and microscopic observations were performed. Similar growth of body mass was observed in the rabbits from both groups during the first two months of the experiment. In month 3 a slightly smaller increase in weight gain was observed in the animals from group 1 than in those from group 2. No morphological lesions were observed in a macroscopic examination. A statistically significant increase in the mean mass of the adrenal glands (0.4253 g ± 0.0033) of the stimulated rabbits in comparison to those from the group 2 (0.2981 g ± 0.0087) was noted. A microscopic examination revealed hypertrophy of zona fascicularis with visible overgrowth of glandular cells in the rabbits from group 1. The results of clinical observations as well as morphological lesions indicate the presence of adaptive stress in rabbits stimulated with ST-LESS.

Key words: rabbits, adrenal glands, scoliosis, short term electrical stimulation, pathomorphology.

Currently, shortened-duration of the daily lateral electrical surface stimulation (LESS) of paravertebral muscles in conservative therapy of idiopathic scoliosis (IS) in children and adolescents is recommended (10, 14, 17, 24, 25). It has been shown in clinical and experimental trials that long-term (9 h daily) and short-term (2 h daily) stimulation has a similar effect on IS correction (14, 15, 18, 24, 25). Moreover, clinical observations indicate that ST-LESS has fewer side effects as compared to those that accompany the method in its extended time (14).

In light of the facts, it is justified to use the available experimental material, i.e. rabbits previously treated with ST-LESS (16), in order to carry out an analysis of their body mass gain during the period of experiment, and a morphological examination of their adrenal glands.

Material and Methods

Investigations were carried out on 10 pure-bred New Zealand male rabbits, aged about 3.5 months with body mass from 2,000 g to 2,200 g. The animals were purchased from a breeding farm OSHZ in Gdańsk (denoted as G-1 in the list of Breeding and Reproductive Rabbit Farms of the Ministry of Agriculture and Rural Development). The animals were characterised in detail and information about the conditions of their breeding and feeding during the adaptation period (30 d) and during the experiment was presented by Kowalski et al. (16).

The rabbits were divided into two equal groups: group 1 - in which the animals were subjected to ST-LESS for 2-h daily during 3 months, and group 2 – a control group, in which the rabbits were not stimulated,
but other experimental parameters were the same as in group 1 (16). Both the technical parameters of the SCOL-2 apparatus (ELMECH, Poland) and the manner in which the electrostimulation was carried out have been described by Kowalski et al. (15, 16).

Clinical observations were conducted every day at the same time and the temperature was measured daily between 8.00 and 9.00 am. Once a month, the animals were weighed. After 3 months of the experiment, the rabbits were sacrificed, and a macroscopic examination was performed. The rabbits’ body mass growth and adrenal gland weights were analysed statistically using Student’s t-test.

Sections of the right and left adrenal glands were taken to the microscopic examination. The material was fixed in 10% buffered formalin and 10 µm paraffin sections were routinely stained with haematoxylin and eosin (HE).

Results and Discussion

ST-LESS group. Hyperactivity was observed in rabbits during the first month of the experiment. The animals were over-active and moved rapidly inside the cages. These symptoms became more intensive in the 2nd month. The rabbits avoided people and moved to distant parts of cages, especially when stimulation was turned on. Symptoms of excitation intensified even more in the 3rd month. Feed was, however, readily consumed throughout the experiment, and amounts of consumed feed did not change. Body temperature measured daily remained at a normal level (from 38.5°C to 39.0°C). The individual mass of animals increased in this group from the mean 2,100 g at 0 period to 2,460 g and 2,600 g after 2 and 3 months, respectively (Table 1).

The macroscopic examination revealed the normal pattern of organs and tissues of the rabbits. The average mass of adrenal glands in the animals of the group was 0.4253 g ± 0.0033. The organs were brown-grey, elastic, with the cross-section pattern typical for the species.

The microscopic examination revealed a well-developed connective tissue capsule surrounding the adrenal glands. From it, the trabecular connective tissue ran inside the gland parenchyma. Underneath there was the zona glomerulosa, formed by the glandular epithelial cells. Separated from it, there was the layer of the zona fasciculata. This layer was considerably widened (Fig. 1). The glandular cells showed symptoms of hypertrophy. With no clear border, they transformed into another group of cells – zona reticularis. The structure was slightly narrowed and had few blood vessels.

Control group. Rabbits behaved normally throughout the experiment. They were interested in their surrounding, both inside and outside cages, and were calm and reacted in a normal way to contact with people. Feed was readily consumed in unchanged amounts. Body temperature was normal and ranged from 38.5 to 39.0°C. Individual body mass increased continuously during the whole experiment, from a mean level of 2,080 g in period 0 to 2,940 g after 3 months (Table 1).

A post mortem examination of the internal organs showed no pathologic lesions. The mean mass of the adrenal glands was 0.2981 g ± 0.0087.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Individual body mass of rabbits subjected to short time lateral electrical surface stimulation (ST-LESS)</th>
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<tbody>
<tr>
<td>Examination period</td>
<td>Mean body mass (g) of rabbits</td>
</tr>
<tr>
<td></td>
<td>ST-LESS group</td>
</tr>
<tr>
<td>A - before the experiment</td>
<td>2,100</td>
</tr>
<tr>
<td>B - after 1 month</td>
<td>2,488</td>
</tr>
<tr>
<td>C - after 2 months</td>
<td>2,640</td>
</tr>
<tr>
<td>D - after 3 months</td>
<td>2,600</td>
</tr>
<tr>
<td><strong>Comparison of mean body mass (Student’s t-test)</strong></td>
<td></td>
</tr>
<tr>
<td>A with B</td>
<td>4.9*</td>
</tr>
<tr>
<td>A with C</td>
<td>7.9**</td>
</tr>
<tr>
<td>A with D</td>
<td>7.9**</td>
</tr>
<tr>
<td>B with C</td>
<td>3.9*</td>
</tr>
<tr>
<td>B with D</td>
<td>1.1</td>
</tr>
<tr>
<td>C with D</td>
<td>0.4</td>
</tr>
</tbody>
</table>

* difference significant at α = 0.05.
** difference significant at α = 0.01.
A post mortem examination of the internal organs showed no pathologic lesions. The mean mass of the adrenal glands was 0.2981 g ±0.0087.

The microscopic pattern of the adrenal glands was normal (Fig. 2). It was surrounded by a well-developed connective tissue capsule and its trabeculae branched into the parenchyma of the organ. Underneath, the glandular epithelial cells made up the structure of the zona glomerulosa. The zona fasciculata with parallel strands of cells running in radial lines towards the medulla, was clearly separated from the zona glomerulosa cells. The glandular cells of the internal part of the layer were of gradually decreasing in size. The number of granules in their cytoplasm, which became increasingly acidophilic, was also reduced. In this way, the layer was gradually being transformed into the zona reticularis. Its glandular cells had smaller nuclei, few granules, and their cytoplasm was more acidophilic.

Stress can have a positive or harmful effect on a living organism, depending on its intensity and duration (1, 6, 9, 21, 23). If the stress parameters exceed the regulatory capabilities of the organism, its effect is positive (19, 20). Many systems and organs are then positively activated (5, 11, 12, 14, 19, 20). Stress, and especially its alarm reaction, is of great importance in organism adaptation to environmental changes as part of organism defence and positive adaptation, which result in adrenalin secretion from the adrenal gland medulla (21).

Adaptive stress was observed in rabbits of the group 1. It manifested itself in the morphological pattern of the adrenal glands. The signs of the organ hypertrophy were found in all the animals subjected to ST-LESS. The mass of their adrenal glands was significantly greater by nearly 30% as compared to such glands in control animals. The hypertrophy was particularly distinct in the microscopic pattern of the zona fasciculata.

In LESS for 2 h daily, emotional stress is created (neurogenic, psychogenic, short-term) by triggering the sympathetetic adrenal medullary mechanism (14). It is a type of stress of positive adaptation, which activates, among other things, the neuromuscular system, which has a corrective effect on IS. (2-4, 7, 8, 10, 12, 13, 22).

In light of the study presented here, it is necessary to continue in-depth studies with a view to shortening daily stimulation in the treatment of low-degree IS in children and adolescents in order to minimise the systemic side effects caused by stress (6, 14, 17, 21). Studies with this aim were started by Kowalski (14). Shortened (lasting only 2 h daily) evening LESS therapy applied did not reduce the expected clinical effects (14, 18, 24). Encouraging preliminary clinical studies, lasting several years and supported by experiments on animals, suggest that continuation of this type of treatment could be justified (6, 14, 18, 24).

The clinical observations, macroscopic examination, and morphological microscopic pattern of the adrenal glands in rabbits indicate that adaptive stress occurred in the animals treated with short-term lateral electrical surface stimulation.

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References