

THE USE OF VARIOUS MODELS OF CHRONIC IMMOBILIZATION STRESS IN EXPERIMENTAL STUDIES

WYKORZYSTANIE RÓŻNYCH MODELI STRESU WYWOŁANEGO PRZEWLEKŁYM UNIERUCHOMIENIEM W BADANIACH EKSPERYMENTALNYCH

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ABSTRACT

Introduction: Stress is defined as a complex of protective and disturbing responses of the organism, generated in the process of the evolution, which occur as a result of neuroendocrine and metabolic alterations in response to the impact of emergency or pathological factors. Hence, stress is an important link of the adaptive mechanism; however, its adverse course can contribute to pathogenesis of many diseases. The problem of stress, which has been under study for years, is still a relevant topic to be investigated.

Aim: The paper was aimed at comparison of various models of chronic immobilization stress based on the published data and findings from our own research.

Materials and Methods: The comparative analysis of the various techniques of chronic immobilization stress simulation in rats has been carried out on the basis of published data and findings of our own studies.

Results: It has been shown that the model, proposed by the investigators from the Ukrainian Medical Stomatological Academy aids in reducing the time of experimental study, does not require sophisticated equipment and the use of additional stressors, at night time, in particular, as well as enables animals' restraint in the convenient morning time.

Conclusions: This technique is easy-to-use and can be applied while studying the influence of the immobilization stress on the body.

KEY WORDS: stress, immobilization, rats.

Wiad Lek 2017, 70, 3, cz. II, 619-621

INTRODUCTION

Stress is defined as a complex of protective and disturbing responses of the organism, generated in the process of the evolution, which occur as a result of neuroendocrine and metabolic alterations in response to the impact of emergency or pathological factors. Hence, stress is an important link of the adaptive mechanism; however, its adverse course can contribute to pathogenesis of many diseases. The problem of stress, which has been under study for years, is still a relevant topic to be investigated [1, 2, 3]. The study of the mechanisms of chronic stress and seek for ways to increase the resistance to it is one of the contemporary topical medical and biological problems. Experimental studies with the use of various stress models are beneficial for contemporary scientists in resolution of these issues.

THE AIM

The paper was aimed at the comparison of the diverse models of chronic immobilization stress based on the published data and the findings from our own research.

MATERIALS AND METHODS

The comparative analysis of the various techniques of chronic immobilization stress simulation in rats has been

carried out on the basis of published data and findings of our own studies.

RESULTS

Numerous experimental models have been developed to study the morphofunctional changes caused by the chronic immobilization stress, e.g., model of stress-induced gastric ulcers [4], when the animals are subjected to single-time immobilization during 5 hours in the stiff cartridges, stored in the freezer at 3-4°C. Stress-related ulcerogenesis has a universal nature and indicates about the imbalance of protective reactions, involving neurohumoral mechanisms. Experimental model is easy to use, but requires specific cartridges to restrain laboratory animals.

In experimental models of chronic immobilization stress immobility can be combined with other techniques: swim test, electric foot shock stress, situational stress, etc. Such experimental models can be more complicated to be performed and require additional equipment.

One of the models assumes additional simulation of the situational stress when, after the period of immobilization, the animals' habitual dietary and watering regimens are violated, animals are kept in the overpopulated cages, contingent of animals is changed under conditions of random sequence

[5]. Experimental model in which situational stress stimulus is intensified by luminous effect at night time and electric current of threshold sensitivity with prolonged duration of the stressors' activity up to 10 months [6] has been proposed to simulate the chronic stress of different degrees in rats. The use of such model extends the time for experiment, requires the researcher to work at night period and application of special devices to generate electric current of the threshold value. One of the advantages of this mode is the possibility to adjust the degree of stress influence.

There is another way to simulate a complex model of chronic stress in adult Wistar rats that combines the vertical rotation, restraint, strapping, night lighting during 6 weeks, sequentially, for 6 hours a day [7]. Animal model mimics the tension in the human body and can be used to study changes in the living body occurred under the influence of stress; however, it is complicated in execution.

Quite simple in execution is a way of simulation of chronic immobilization-emotional stress by restraining the rats daily in the morning for 3-4 hours during 55-60 days. Animals are placed in tight-fitting wire mesh restrainers in groups of three where they are in close contact. Then the blocks of each 7-9 wire mesh restrainers are composed so that the rats feel the presence of animals in the adjacent restrainers [8].

Similar to the previous model is the model of chronic immobilization stress, consisted of daily immobilization of the laboratory animals in special wire mesh restrainers at different time intervals for 60 days every day except Sunday. Rats are placed in the individual wire mesh restrainers, composed in blocks for 5 animals so that each rat will be in close contact and feel the presence of neighbor animals. At the end of immobilization period of varying duration, additional situational stress is carried out by the violation of animals' habitual dietary and watering regimen, changes in the composition of animals in standard cages during feeding and watering under conditions of a random sequence [9].

The main disadvantages of these techniques, in our opinion, is a need in special wire mesh restrainers, inadequate unmanageable immobilization of animals and a longer time required for experiment.

To improve the technique of chronic immobilization stress simulation, our own experimental model has been proposed at the Department of Operative Surgery and Topographic Anatomy of the Higher State Educational Establishment of Ukraine "Ukrainian Medical Stomatological Academy". According to it, rats one by one are immobilized by extremities, not squeezing the vessels, in a position lying supine daily in the morning at the same time on an empty stomach for 40 minutes during 21 days. Once the periods of immobility finished, rats are kept in standard conditions of the vivarium in a cage in groups of five on the full diet [10]. The experimental model is easy-to-use, does not require sophisticated equipment and reduces the time to carry out the experiment.

DISCUSSION

Among the various experimental models of chronic immobilization stress, the technique, proposed by the

investigators from Ukrainian Medical Stomatological Academy has a number of advantages: it is simple in execution, does not require application of additional stressors and is conducted in a relatively short time. The effectiveness of the experimental model has been shown in the study of morpho-functional changes occurred in the lungs of white Wistar rats induced by chronic immobilization stress. It was applied to twenty 8-10 month old Wistar white male rats of 240-260 g body weight (experimental group). 20 similar intact animals were assigned to the second (control) group.

The rats were killed on an empty stomach by decapitation under intraperitoneal thiopentone anesthesia on day 22 of the experiment. The experiment was performed in compliance with the requirements of international principals of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1985) and corresponding Law of Ukraine "For the Protection of Pet Animals" (No.3446-IV, 21.02.2006, Kyiv) [11, 12].

After dissection of rats' chest a macroscopic study of its organs has been carried out. Animals which were exposed to the influence of the experimental chronic immobilization stress had significantly enlarged, swollen lungs, edges of which covered the anterior mediastinum. The lungs felt soft by touch and did not collapse after removal from the chest. Crackle and outflow of mucoid exudate was noted during the dissection of the lung tissue. Bronchi were enlarged and filled with clear mucoid exudate.

During the macroscopic analysis no apparent changes in lungs have been found in rats of control group.

Microscopic analysis of histological specimens of lung tissues stained with hematoxylin-eosin has shown a significant enlargement of lumen of the alveoli in rats, exposed to the experimental chronic stress. Alveolar walls were plethoric, malformed and sinuous-shaped due to overgrowth of connective tissue. Locally, alveolar walls were completely destroyed with the formation of conjugation between the adjacent alveoli. Apparent plethora and spasm of arterioles, lesions of their walls with perivascular macrophage infiltration, foci of massive hemorrhages into intersticium, alveolar walls and cavities has been detected. The study of the semi-thin sections of lung tissue, stained with toluidine blue showed the crooked duct of arterioles' elastic membranes due to manifested vasospasm. The phenomena of red blood cells dysdiemorrhysis with the formation of microthrombi in the lumina of venules have been observed in the lumina of vessels. The bronchial tubes lumina was narrowed due to bronchospasm. The areas of damages, inflammatory macrophage infiltration, hemorrhages and thickening of the mucosa have been detected in their walls. Mucous membrane of bronchial tubes was thickened due to the overgrowth of pseudostratified epithelium induced by the pronounced cellular proliferation in response to prolonged bronchospasm. In addition, numerous areas of atelectasis which are microscopically defined by alveoli collapse with no lumina on the slide mounts have been found in the lungs in response to prolonged bronchospasm in the lungs.

No significant morphological changes have been noted

in the animals of control group.

The study showed that chronic immobilization stress affects the lungs of Wistar rats, and the proposed simulation model can be widely used for conducting experimental studies on the impact of stress on the body [13, 14, 15, 16, 17].

CONCLUSION

The model, proposed by the investigators from the Ukrainian Medical Stomatological Academy aids in reducing the time of experimental study, does not require sophisticated equipment and use of additional stressors, at night time, in particular, and enables to restrain the animals in the convenient morning time. This technique is easy-to-use and can be applied while studying the influence of the immobilization stress on the body.

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Received: 08.02.2017

Accepted: 11.05.2017