

## **EXPERIMENTAL RESEARCH INTO THE EFFECT OF HYPERBARIC EXPOSURES**

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### **ABSTRACT**

Numerous research works indicate that staying in a hyperbaric environment is a stressor. We have undertaken studies using an animal model to determine the effect of hyperbaria on adrenocortical secretion and the level of adrenal ascorbic acid (a.a.a.). The research was conducted on 70 male hooded rats, which were divided into groups and subjected to hyperbaric air and oxygen at an overpressure of 1 and 3 atm, with some of the animals being additionally burdened with physical effort (swimming).

It was found that short-term exposures (30 minutes) of rats to hyperbaric conditions caused a decrease in the content of a.a.a. With a prolonged (3-hour) exposure to 3 atm air the a.a.a. content returns to a normal level. A reduction in the a.a.a. content indicates the presence of stress in hyperbaric conditions in the rat; however, it does not allow to determine its intensity with the method used in the study.

**Keywords:** overpressure, animal model, stressor, ascorbic acid.

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### **ARTICLE INFO**

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PolHypRes 2017 Vol. 61 Issue 4 pp. 43 - 48

**ISSN:** 1734-7009 **eISSN:** 2084-0535

**DOI:** 10.1515/phr-2017-0021

Pages: 6, figures: 2, tables: 1

page **www of the periodical:** [www.phr.net.pl](http://www.phr.net.pl)

**Typ artykułu:** oryginalny

Original article

Originally published in Gdańsk Rocznik Naukowym 1969

Acceptance for print inPHR 19.08.2017r

**Publisher**

Polish Hyperbaric Medicine and Technology Society



## INTRODUCTION

It is believed that hyperbaria may constitute a stress factor on an equal footing with other stressors such as cold, noise, vibrations or emotional stimuli [1,3,4,10]. As a result of hyperbaria and the occurred alarm reaction, an increased activity of the pituitary-adrenocortical may be noted alongside an increase in the adrenocortical secretion and a reduction in the concentration of adrenal ascorbic acid (a.a.a.). The adrenal cortex in rats mainly secretes corticosterone [5] the biosynthesis of which requires the presence of ascorbic acid, that is why its concentration in the adrenal glands decreases as the production of cortical hormones increases.

Depending on the trigger, the decrease in the amount of a.a.a. occurs at varying rates, with its degree being proportional to the intensity of stress. Because changes in the concentration of a.a.a. are believed to constitute a specific and quantitative assessment of the activation of the pituitary-adrenal axis under acute stress [12,14,20] we used the a.a.a. determination in hyperbaric air and oxygen conditions as a measure of system burden.

## MATERIAL AND METHODS

The study was conducted on 70 hooded rats, uniform males with the weight of 180-220 g. The animals were kept continuously in a temperature of approximately 24°C and fed ad libitum with a standard

diet and water. Animals used in the study were subjected to hyperbaric conditions while at rest or being exposed to exhaustive physical exercise (swimming test) [7,13,14]. The tests were carried out in an experimental pressure chamber for animals, which was filled with air with the pressure raised to 1-3 atm. The chamber ventilation allowed to keep the CO<sub>2</sub> concentration at a sufficiently low level (0.3%). Animals remaining at rest were subjected to air overpressure equal to 1 and 3 atm for the period of 30 minutes and 3 hours. The swimming animals remained under a pressure of 1 and 3 atm for the period depending on their exercise capacity: 40 minutes for 1 atm and 20 minutes for 3 atm.

The methodology for determining a.a.a. levels was described in a work previously published in "Medycyna Pracy" [13]. The control group consisted of rats that spent 1-3 hours in the experimental chamber at rest without the overpressure being applied.

## RESULTS

The obtained results are presented in Table 1 and fig. 1 and 2.

Tab.1

Ascorbic acid content (a.a.) in adrenal glands of rats in hyperbaric air conditions.

No.	Experimental conditions	Number of rats	a.a. (mg)	Standard error	t <sup>x</sup>	Weight of adrenal glands	Standard error	t <sup>x</sup>
						100g of adrenal glands		
1	Control group xx	18	369±75.2	17.9	-	17.6	0.28	-
2	1 atm 30' rest	12	253±50.6	14.6	5.02>t	18.2	1.26	0.385< t
3	1 atm 3 <sup>h</sup> rest	9	305±50.3	16.7	2.61>t	17.1	0.27	0.397< t
4	3 atm 30' rest	8	250±62.4	20.9	4.34>t	17.7	0.29	0.077< t
5	3 atm 3 <sup>h</sup> rest	8	387±79.8	28.2	0.53<t	17.7	1.26	0.069< t
6	1 atm ca.40' effort	7	294±27.5	10.4	3.62>t	17.4	0.23	0.198< t
7	3 atm ca. 20' effort	8	315±62.0	21.9	1.91<t	16.1	0.26	1.23< t

x – statistical variability in the Student's t test

xx – the animals placed in conditions analogous to the rest of the group, with the exception of overpressure

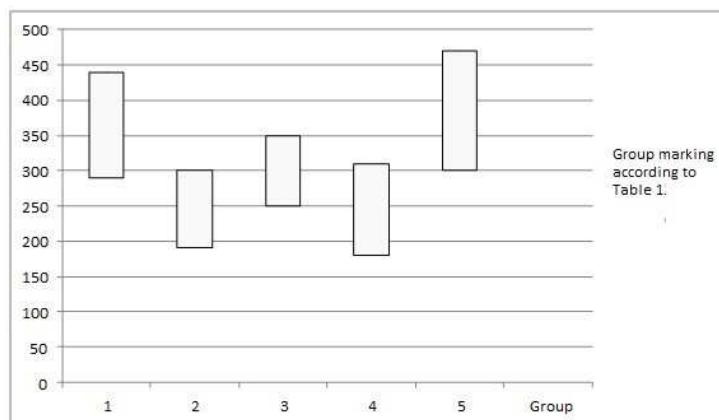


Fig. 1 The a.a.a. content of rats in the rest groups (in milligrams per 100 g of adrenal glands taking into account the average error of the arithmetic mean).

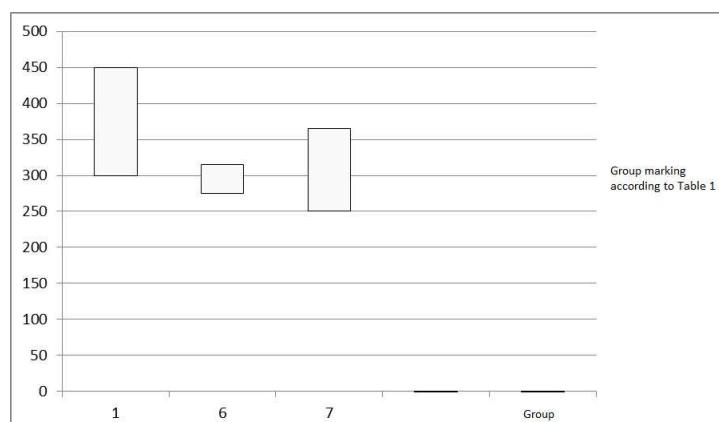


Fig. 2 The a.a.a. content of the rats in groups burdened with physical effort (see above).

In the rest groups (groups 2 and 4 in Fig. 1) exposed for 30 minutes to a pressure of 1 and 3 atm, a statistically significant reduction was observed, while with the exposure time of 3 hours (group 3 in Fig. 1) – at a pressure of 1 atm, a decrease was observed, and at 3 atm (group 5), the a.a.a. concentration did not differ from normal (group 1). In the group burdened with effort for 40 minutes at 1 atm (group 6 in Fig. 2) a decrease was observed – the a.a.a. remained within the normal range.

## DISCUSSION

Research into the a.a.a. level in rats subjected to exhaustive effort, without the application of hyperbaric conditions [14,15] showed its reduction as late as 75 minutes into the exposure or when exhausted, which occurred on average after the lapse of 200 minutes with the a.a.a. being then decreased by approximately 50%. However, in our studies the application of hyperbaria of 1 atm for 30 minutes demonstrated a clear decrease in the a.a.a. Our previous studies [7] demonstrated that hyperbaric oxygenation produced a similar reduction even when the swimming time is significantly shorter.

If we take into account the fact that the test groups differed from the control groups only by the effect of hyperbaria or the type of breathing mixture used, while the overpressure was the same and that the decrease in a.a.a. was observed already during a short-term exposure, we may assume that this change occurred as a result of a stress reaction in hyperbaric conditions. Although it is difficult to assess the stress intensity on the basis of the results obtained for a.a.a., as was suggested by different

authors [6,11,12,15,16,17,19,20], it is also impossible to reject, without further research, the theory that hyperbaria acts as one of the stressors and the observed decrease in the a.a.a. may be one of the symptoms of a general alarm reaction.

When testing the impact of hyperbaria alone, without the imposed physical effort, the results obtained by other authors were not conclusive. Aschan [1] stated that breathing with oxygen at 1 atm after only 2 hours gives a significant decrease in a.a.a., which was not confirmed by the studies of Gerschman and Fenna [9,10], according to which such a drop occurred only after 2-3 days of poisoning. Subjecting the animals to hyperbaric conditions at 5-6 atm for a short period of time resulted in a greater reduction of a.a.a. when breathing with air rather than oxygen, which they associate with the pressure stress. Our tests conducted with air pressures of 1-3 atm, and in the previous work also with the use of pressurised oxygen [13], did not confirm these data, as the drop in both cases was similar. Nonetheless, the application of longer hyperbaric exposures allowed to partially differentiate the effects of these factors.

In the group subjected to air at 3 atm the a.a.a. content, which reduced after 30 minutes spent in hyperbaric conditions, returned to a normal level during the 3-hour exposure. An explanation of this fact requires further research. In the group subjected to hyperbaric oxygenation at 3 atm for 3 hours, the a.a.a. content remained at an equally low level as at 30 minutes [12]. The fact that seems difficult to explain is that at 3 atm of air after 20 minutes of effort, the level of a.a.a. was normal. Perhaps the exposure time was too short [20] minutes) to note a significant decrease in the content of



a.a.a. [12, 20]. Moreover, only a single stress factor was present in the form of hyperbaria without an additional influence of oxygen.

One could assume that as far as the drop in a.a.a. during short-term exposures depended on pressure stress, a prolonged exposure allowed to reveal the specific effect of hyperbaric oxygenation [10]. This is consistent with the hypothesis [16,17] that corticoadrenal secretion may remain high throughout the time of the stressor's activity if an animal is unable to adapt to it.

Hyperbaric oxygenation constitutes a stress that is not found in regular phylogenetic development, and the theory that an increase in the secretion is a purely defensive phenomenon cannot be accepted without a reservation. Bean [3,4] affirmed that the elimination of the cortical-adrenal axis protects an animal against the effects of hyperbaric oxygenation, whilst any conditions that increase tissue oxygen consumption, which occurs, inter alia, in the case of an increased secretion of adrenal

hormones, shortens the survival time of an animal. It is possible that hyperbaria, and in particular hyperbaric oxygenation, constitutes a strong stress factor which, especially in unfavourable conditions, reveals its effects even within the pressure range assumed to be inert in the body [18].

## CONCLUSIONS

1. It was found that a short-term exposure (30 minutes) of a rat to hyperbaric conditions causes a decrease in the content of a.a.a.
2. With a prolonged (3-hour) exposure to air at 3 atm the a.a.a. content returns to a normal level.
3. A reduction in the a.a.a. content indicates the presence of stress in hyperbaric conditions in the rat, however, it does not allow to determine its intensity with the method used in the study.

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