THE EFFECT OF BREATHING MIXTURES ON PHYSICAL CAPACITY OF RATS IN HYPERBARIC CONDITIONS

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ABSTRACT

The purpose of the study was to determine the effect of composition of various breathing mixes on physical capacity of rats swimming in hyperbaric conditions. The said effect was determined on the basis of results of a swim test performed in a pressure chamber. The study was performed with the use of atmospheric air, a mixture of nitrogen and oxygen ($N_2/0_2$) at a ratio of 89.5/10 and 92/7.5, as well as a mixture of argon and oxygen at a ratio of 79.5/20 ($Ar/0_2$). The tests were conducted at a pressure range between 0-4 atm. The results suggest that the physical capacity of the tested animals decreased along with pressure increase regardless of the breathing mix used. Due to the fact that the burdening of rats with physical effort in hyperbaric conditions intensifies the adverse effects of components of breathing mixes on their performance, it seems appropriate to continue the study of physiological responses to breathing mixtures of various compositions in human body subjected to physical effort while under water.
Keywords: mixed gas, hyperbary, effort, rats.

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INTRODUCTION

A high interest in long-term underwater stays, whether for commercial, recreational or sports-related purposes, raises the need for experimental research aimed at determining the factors which limit the physiological capabilities of humans to operate in such conditions. Research in this area is rarely undertaken due to the hazard of poisoning with components of a breathing mix as well as other restrictions regarding research on humans. As a consequence, a significant part of research on the discussed subject area is carried out on animals [1,2]. Therefore, as in previous own studies [3], the study was carried out on rats which were subjected to a swimming test until exhaustion. Wilberg [4] explains the diagnostic value of the test as follows: "... *if a substance of an unknown characteristic is administered to a mouse, the change in the swimming time until exhaustion can be regarded as a determinant of the adverse effects of this substance*."

The same author, as well as other researchers [5,6] recognise the use of an exhaustive swimming test as purposeful in studies of the environmental impact on animal organisms as the spread of swimming times of animals of similar weight and at a particular temperature is minor [4,6,7,8]. Therefore, an interpretation of this type of studies may be used to formulate application trends allowing to draw conclusions regarding the response of the human body to a given breathing mix, without the need to expose it to the consequences of undertaking maximum effort in adverse conditions of a high pressure, humidity and low temperature. Indeed, it is a well-known fact that in the conditions of a maximum burden with physical effort the harmful effects of various factors of ambient environment are observed earlier and the reaction of the body is stronger [1,9,10,11,12,13,14].

OBJECTIVE

The purpose of the study was to determine the effect of the composition of various breathing mixes on the physical capacity of rats swimming in hyperbaric conditions.

RESEARCH METHODS

The study was conducted on 131 hooded male rats, with the weight of 250-300 g, on a standard diet and ad libitum water.

The hyperbaric swim test was performed in an experimental pressure chamber designed for small animals. The animals swam until exhausted. The criterion of exhaustion was the cessation of movements in a submerged animal for the period of 30 seconds. The construction of the chamber and the water level prevented the animals from resting or supporting themselves with a tail or feet while swimming. Upon test completion the animals were subjected to decompression. The procedure of the experiment was positively assessed by the relevant Committee on Ethics in Science.

Tab. 1

Physical properties of breathing mixtures used in the study.

pressure	depth equivalent	Air					Nitrox					Argon mix				
		composition %		O2 partial pressure	1 litre weight	density in reference to air at 0 atn	composition %		O2 partial pressure	1 litre weight	density in reference to air at 0 atn	composition %		O2 partial pressure	1 litre weight	density in reference to air at 0 atn
[atn]	[m]	[O2]	[N2]	[mm Hg]	[g]		[O2]	[N2]	[mm Hg]	[g]		[O2]	[N2]	[mm Hg]	[g]	
0	surface	20.95	78.05	159	1.29	1	-	-	-	-	-	-	-	-	-	-
1	10m	20.95	78.05	318	2.58	2	10%	89.5%	152	2.53	1.95	20%	79.5%	306	3.42	2.64
2	20m	20.95	78.05	477	3.87	3	7.5%	92%	171	3.79	2.92	20%	79.5%	459	5.14	3.98
3	30m	20.95	78.05	636	5.17	4	7.5%	92%	229	5.06	3.91	20%	79.5%	612	6.85	5.29
4	40m	20.95	78.05	795	6.46	5	7.5%	92%	286	6.33	4.9	20%	79.5%	765	8.56	6.61

In all trials, the animals were subjected to the same experimental procedure. Once they were placed in the chamber for the first time, the chamber was ventilated with a considerable excess of air for two minutes and then filled with gas mixtures. The gases used in the research were:

- air,
- hypoxic nitrox 10 (nitrogen/oxygen mixture at a ratio 89.5/10) for an experiment with the overpressure of 1 atm,
- hypoxic nitrox 7.5 (nitrogen/oxygen mixture at a ratio 92/7.5) for an experiment with the overpressure of 2, 3 and 4 atm,
- argox 20 (mixture of argon and oxygen at a ratio 79.5/20).

The physical properties of mixtures used are given in Table 1.

In total 131 exposures were performed:

- 52 with air,
- 43 with nitroxes,
- and 36 tests with the use of argon mixture.

Animals were exposed to high pressure:

- ranging from 0 to 4 atm for air,
- ranging from 1 to 4 atm for other mixes.

The pressure in the chamber was raised at a rate of 0.5 atm/min.

The animals swam in a tank filled with water at the temperature of 24°C. During all exposures, the chamber was ventilated with a particular breathing mix in the quantity of approximately 10 l/min, with CO_2 level being controlled. This allowed to maintain the level of carbon dioxide at a sufficiently low level to ensure that its concentration would not affect the physical capacity of the animals. During each test samples of the mixture used were collected from the chamber and analysed with the use of a gas chromatograph (Willy Giede G-CH 21 by DDR). The extraction of the gases used /oxygen, nitrogen and argon/ was carried out on a molecular sieve using hydrogen as the carrier gas.

RESULTS

Tab. 2

Summary of average swimming times until exhaustion (in minutes) for the whole group of tested animals in a given gas mixture and in defined pressure conditions.

Type of the breathing mix												
pressure	no. of tests	Air			no. of tests	Nitrox			no. of tests	Argox		
[atn]		t ($\bar{x}$)	σ ($\bar{x}$)	Σ ($\bar{x}$)		t ($\bar{x}$)	σ ($\bar{x}$)	Σ ($\bar{x}$)		t ($\bar{x}$)	σ ($\bar{x}$)	Σ ($\bar{x}$)
1	11	39	10.5	3.31	10	33.4	6.9	2.21	9	16.4	1.9	0.64
2	20	20.9	3.9	0.89	13	21.3	6.2	1.72	11	11	2.4	0.73
3	11	17.8	7.7	2.33	10	18.7	8.1	2.57	8	6.1	1.2	0.44
4	10/52	13.6	3.0	0.97	10/43	17.6	4.4	1.39	8/36	5.7	1.6	0.59

The results presented in table 2 indicate average swimming times until exhaustion $t(\bar{x})$ (in minutes) for the whole group of tested animals with the use of a given gas mixture and in defined pressure conditions. The table also provides values of standard deviation $\sigma(\bar{x})$ and standard error $(\bar{x})$as well as specifies the number of tests performed in specific conditions. According to the provided data, along with an increase in pressure the times obtained by the animals while swimming until exhaustion were shorter, irrespective of the breathing mix used. The animals swam for the longest period of time in the air atmosphere, shorter while breathing with the nitrogen mixture, and the shortest time in the atmosphere of the argon mix. The statistical significance of differences was noted between the times of swimming while breathing with air at the atmospheric pressure and the times of swimming in hyperbaric conditions with the use of argon mix and air, as shown in Table 3.

Tab. 3

The results of the difference significance test (t-Student) between average swimming times $t(\bar{x})$ (in minutes) for the entire group of tested animals with the use of a given gas mixture and in specified pressure conditions.

air : air	air : nitrox	air : argox
0 atm : 1 atm : 5,26 > t	1 atm : 1 atm : 1,40 > t	1 atm : 1 atm : 6,68 > t
2 atm : 3 atm : 1,26 > t	2 atm : 2 atm : 0,18 > t	2 atm : 2 atm : 8,0 > t
3 atm : 4 atm : 1,66 > t	3 atm : 3 atm : 0,25 > t	3 atm : 3 atm : 4,9 > t
	4 atm : 4 atm : 2,35 > t	4 atm : 4 atm : 6,9 > t

indicates the pressure in absolute atmospheres in a logarithmic scale, whilst the ordinate axis indicates swimming times in minutes starting at 2 atm, which isassumed to be 100%. Further percentage values indicate a deterioration of results in hyperbaric conditions as compared to the baseline.

Fig. 2. shows the values of the obtained swimming. The control group of rats swimming in the atmosphere of air pressure at sea level always produced the result of over 60 minutes.

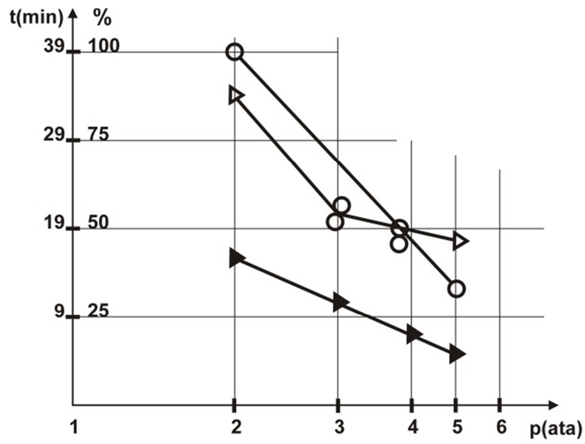

Fig.1 The obtained swimming times in particular animal groups breathing with different breathing mixes - air, nitrox, argox.

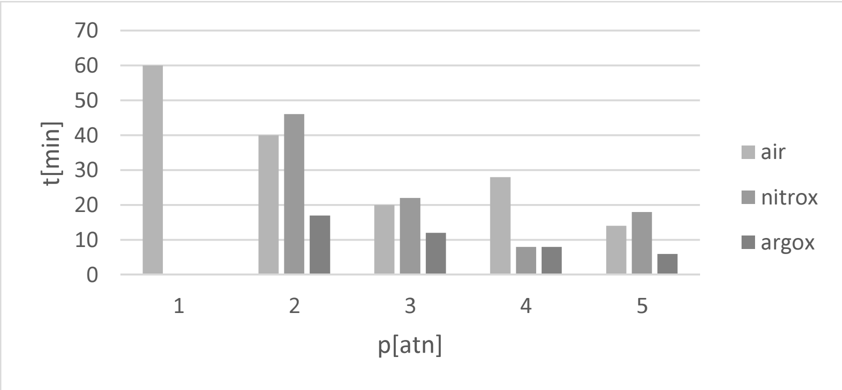

Fig. 2 Swimming times obtained while breathing with particular mixes.

DISCUSSION

When taking up the study it was assumed that the physical capacity of animals under hyperbaric conditions mainly depends on respiratory efficiency [14]. With the assumption that minute ventilation in a man decreases almost proportionally to the density of a breathing gas [16,17,18], it is also possible to investigate the effect of mixtures of different compositions on the respiratory system. Although it is known that the density of gas flowing through the bronchial tree is one of the determinants of respiratory resistance [16,17,18], little research has been devoted to investigating the effect of density of a breathing mix on physical capacity in the conditions of burdening a subject with effort (including extreme effort) [19].

The impact of an increase of respiratory resistance on physical capacity can be studied by modifying the density of a breathing mix (its composition) or by changing the pressure in the working environment [18]. Such a procedure was applied in our research. We used air and nitroxes with a slightly increased density and an argon-oxygen mixture with relatively highest density. As it was demonstrated (Table 2), the exhaustion of animals while swimming in the atmosphere of an argon mixture occurred much earlier as compared with animals breathing with air. Such a result was obtained in all tested pressure ranges (1-4 atm). Other studies conducted at pressures raised up to 1 atm [20] also showed a deterioration in the physical capacity.

Breathing with mixtures with the density greater than air leads to a relative weakening of the efficiency of the respiratory tract and, consequently, to a reduced exercise capacity of the organism [1]. A highly intense effort in such conditions involves the risk of CO_2 retention, respiratory acidosis, and leads to organism exhaustion, while the continuation of an effort of moderate intensity can help maintain homeostasis [19]. It can be assumed that the physical stimuli experienced by the animals during the described experiment were above the regular threshold and the rats deprived of the capacity to regulate their behaviour in extreme conditions quite quickly experienced complete exhaustion. To compare, the swimming time at the pressure of 4 atm while breathing with air was 13 minutes and only 5 minutes when the breathing mix was based on argon (Table 2).

The study did not take into account the effect of inert gases on the animals. The relationship between the physical properties of such gases and effects of their biological activity are recognised [7,20,21,22]. Based on the results of this study it appears that within the applied pressure range /up to 4 atm/, the narcotic effects and toxicity of the mixtures used were not revealed. Even a shorter swimming time of animals breathing with nitrox, where the oxygen content was lowered to 7.5 or 10% as compared to the swimming time while breathing with air (at 2-4 atm) (Table 2) could indicate a favourable effect of high $p0_2$ in hyperbaric conditions.

The results that are available in the study area [1,10,16,17,18,20,23,24,25,26,27] only allow to make selective references to the results of this experiment, hence the generally reduced physical capacity of the animals in the experimental conditions is greater than that in the cited works. This can be explained by particularly unfavourable conditions in which the study was conducted (high humidity, effort until exhaustion, coldness, stress), which, however, were very reminiscent of the conditions of diver's work.

These animals did not reveal deviations in $p0_2$ and pCO_2 in blood, however they showed subjective symptoms of nitrogen narcosis. Bennet's studies [20]. Whereas Bennett's study, performed at a pressure of 2.4 atm suggested the greatest increase in pCO_2 in the brain when breathing with argon mixture. The level of pCO_2 while breathing with nitrogen mixture was only slightly higher than during breathing with pure oxygen. The result obtained in the experiment of breathing with argon mixture is associated with disorders in the respiratory mechanics resulting from the high density of this mixture. In our study, the $Ar/0_2$ mixture at 4 atm was 6.61 times denser than air, and it appears that in a long-term physical activity it would lead to respiratory failure [5,7,21,22].

Although studies on the factors limiting the stay and survival of living organisms in hyperbaric conditions have not yet provided answers to numerous important questions, it seems that today's tendency to conduct research on the toxic effects of particular components of breathing mixes should pave the way towards an exploration of the effect of density on the physical capacity of living organisms.

Conclusions

1. The physical capacity of the test animals decreased along with pressure increase, regardless of the breathing mix used.
2. Breathing with argon mixture reduced the physical capacity of the tested animals significantly more visibly as compared with breathing with nitrogen mix and air.
3. The maximum intensity physical effort made by rats in hyperbaric conditions strengthens the adverse effect on an organism of the components of breathing mixes used during the dives.

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