METHOD OF STUDYING THE EFFECT OF DRUGS ON WORKING CAPACITY OF ANIMALS IN HYPOBARIC HYPOXIA

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An urgent problem in modern pharmacology is the creation of drugs to stabilize or enhance physical working capacity under unfavorable conditions [2-4, 6] and to study the effect of known drugs on resistance of the body to physical exertion, undertaken under complicated conditions [7]. One of the main pathophysiological phenomena reducing the intensity and duration of working capacity is a deficient oxygen supply to the organs and tissues [3, 11, 14]. The antifatigue effects of drugs and of biologically active substances therefore are manifested as a positive effect on physical working capacity under hypoxic conditions [3]. The tissue–blood model of hypoxia, created by means of sodium nitrate [3], and used for this purpose, is inadequate and is rarely used in practice. The known method of seeking antifatigue drugs for use in hypobaric hypoxia envisages working on a treadmill, located in a pressure chamber [3], but this is complicated in use. The aim of the present investigation was to develop a simple and informative method of demonstrating the antifatigue action of new chemical substances or to study the effect of known drugs on working capacity under complicated conditions.

EXPERIMENTAL METHOD

The suggested method is based on determination of the action of the test substances on the duration of swimming by animals at an altitude of 4000-6000 m. The investigation was conducted on an apparatus shown diagrammatically in Fig. 1. The animals swam in a pressure chamber [1] made of transparent plastic (dimensions of the chamber 60 × 60 × 60 cm, thickness of its walls 1 cm). The pressure chamber was filled with water heated to a temperature of 25-30°C. Air was withdrawn from the chamber through a special pipe [2], by means of a VN-361 vacuum pump [3]. The rate and absolute degree of elevation of the animals were judged by means of an airplane altimeter [4], connected to the pressure chamber. The rate at which the animals were raised and the absolute elevation and air exchange were regulated by means of the control valve 6. In the experiments of series I the duration of swimming by mice (weighing 18-20 g, with an initial load of 5% of body weight, fixed to the tail) was studied at an altitude of 4000 m (rate of elevation 2000 m/min). It was found that at an altitude, air is extracted in abundance from the water and adsorbed in the form of bubbles on the animals' fur, keeping them afloat (the mice cease to work and are held on the surface of the water like floats). With an increase in the load (from 5 to 15%) the animals recommence work, but the length of time they are able to swim under these circumstances is short (from 0.1 to 1.5-2 min), and the data show considerable scatter. The use of boiled water as the medium for swimming or smearing the animal with goose fat reduced the extraction of air from the water and limited adsorption of air bubbles on the fur during elevation to an altitude, but there were sufficient bubbles to keep the animals afloat. In the experiments of series II (to reduce the area of the animals' hair cover with adsorbed air bubbles) the working capacity of rats was therefore studied during swimming at an altitude of 4000 m, in the antiorthostatic position. For this purpose noninbred albino rats weighing 180-200 g were fixed by the tail to a bar 1 by means of a vacuum hose so that the animals' head and neck were immersed in the water (Fig. 2). This position, according to data in [1], compels the animals to hold the head above the water surface by dynamic physical activity (by paddling movements of the forelimbs) during the action of an antiorthostatic load (Fig. 2b). The intensity and duration of work of the rats was controlled by means of a weight (8% of body weight), fixed to the animals' neck. Under these conditions air bubbles adsorbed on the fur of the forelimbs,

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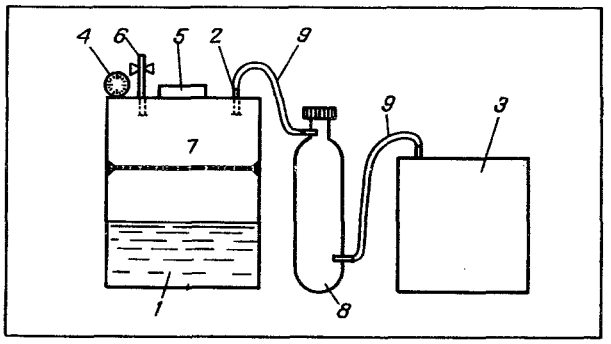

Fig. 1. Diagram of apparatus for studying working capacity under conditions of hypobaric hypoxia. 1) Pressure chamber, 2) pipe for withdrawing air, 3) VN-361 vacuum pump, 4) airplane altimeter, 5) port of pressure chamber, 6) control valve, 7) bar for fixing animals in antiorthostatic position, 8) reservoir for steady withdrawal of air, 9) vacuum hose.

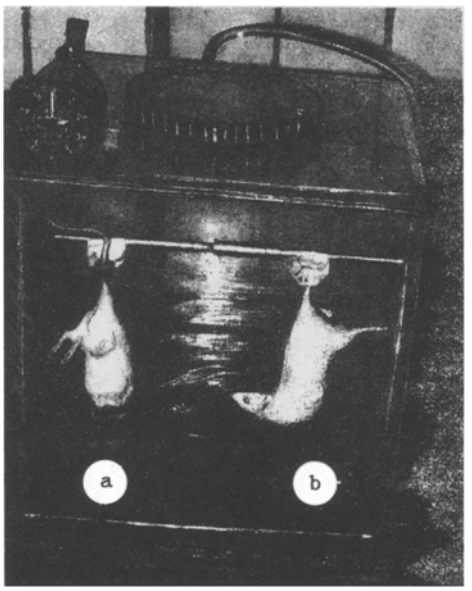

Fig. 2. General view of apparatus for studying working capacity of animals during exposure to hypobaric hypoxia (4000 m). a) Rat drowned after muscular fatigue, b) through intensive muscular activity animal holds its head above the surface of the water.

neck, and chest had virtually no effect on the animals' work. The duration of swimming of the animals at normal atmospheric pressure varied from 10 to 18 min, but after elevation to an altitude of 4000 m, from 3.6 to 7.2 min. To determine the adequacy of the suggested method, the effect of the following drugs or biologically active compounds, stimulating working capacity, on the ability of the rats to work in an atmosphere with the normal oxygen concentration and after elevation to a high altitude: the sympathominetic amphetamine [3], the antifatigue agent bemitil [3, 7], the antioxidant ionol [9, 10], the substance mildronate, accelerating recovery of working capacity [12], and the adaptogen AKS-85 [8, 13]. The substances tested — bemitil, amphetamine, AKS-85, and mildronate were injected intraperitoneally into the animals or were given into the stomach by means of a special tube 1 h before swimming; ionol was administered to the animals for 3 days (once daily, the last dose 1 h before physical exercise). The experimental results were subjected to statistical analysis by Student's test [5].

TABLE 1. Effect of Amphetamine, Bemitil, Mildronate, Ionol, and Compound AKS-85 on Duration of Swimming by Rats in Antiorthostatic Position and at Normal and Reduced Oxygen Concentrations (4000 m; $M \pm m$)

Substance	Amphetamine	Mode of administration	Duration of swimming					
			under normal conditions			at altitude of 4000 m		
			control, min	expt., min	per cent of control	control, min	expt., min	per cent of control
Amphetamine	1	Intraperitoneally	16,5±0,3	22,3±0,3*	35,1	3,6±0,3	1,6±0,5*	55,5
Bemitil	50	Intragastric route	10,5±0,2	14,5±0,4*	38,1	6,5±0,2	6,3±0,1	3,1
Mildronate	5	» »	10,3±0,7	15,6±2,1*	51,4	7,2±0,7	8,0±0,5	11,1
	20	» »	10,3±0,7	20,5±2,2*	99,0	7,2±0,7	9,0±0,2*	25,0
Ionol	20	» »	18,1±0,4	26,1±0,6	44,1	4,8±0,4	6,6±0,4*	37,5
Compound AKS-85	20	» »	10,6±0,8	10,6±0,3	0	5,1±0,4	10,7±0,3*	109,8
	50	» »	10,6±0,8	18,6±1,3*	75,5	5,1±0,4	12,8±0,7*	150,9

Legend. Asterisk indicates statistically significant differences ($p < 0.05$) compared with control.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that the duration of work of the animals at a height of 4000 m was reduced by 2.5-4 times compared with work at a normal atmospheric pressure, in agreement with data in the literature [3]. The test substances, under normal conditions, increased the duration of swimming by the animals until they were completely fatigued in the antiorthostatic position, but when the oxygen concentration was lowered, the substance depressed (amphetamine), did not change (bemitil), or increased (mildronate, ionol, AKS-85) the duration of swimming by the rats. This reversal of the action of amphetamine on the working capacity of animals exposed to hypobaric hypoxia can be explained on the basis of data obtained by Pastushenkov [3], who found that amphetamine reduces the resistance of the body to hypoxia because of acceleration of oxygen-dependent metabolic processes. The antifatigue action of the antioxidant ionol is of definite interest. It can be tentatively suggested that activation of peroxidation during intensive work takes place in the body not only at a normal air pressure [11], but also during work under conditions of hypobaric hypoxia. Hence the need to continue the search for antioxidative compounds. The marked stimulating action of the compound AKS-85 on working capacity, especially under hypoxic conditions, can probably be explained by its antioxidant and antihypoxic effects [13]. Unlike its action under normal conditions, mildronate led to a very small increase in the duration of swimming by the animals at an altitude. The antifatigue action of mildronate can probably be explained by its inhibitory action on the formation of detergent metabolites of fatty acids and by stabilization of the intracellular calcium exchange during stress and hypoxia [12].

The suggested method of studying the effects of biologically active substances on the physical working capacity of animals exposed to hypobaric hypoxia is thus easy to use and yields adequate data. The method can be used to study physical and chemical factors and their effects on the state of working capacity of small laboratory animals, and in the search for antifatigue agents, or to determine the effect of known drugs used under appropriate pathological conditions on working capacity at high altitudes.

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EFFECT OF DIAZEPAM AND OF N^6-CYCLOHEXYLADENOSINE ON LEVEL OF DIAZEPAM-BINDING INHIBITOR IN THE HIPPOCAMPUS DURING IMMOBILIZATION STRESS

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Adenosine and its stable derivatives possess antistressor and anticonvulsant activity [4, 5]. The physiological effects of this group of preparations have been shown to take place through a system of specific adenosine receptors of the A_1- and A_2-types, located on presynaptic membranes of neurons [8]. Meanwhile, some investigators have shown that purine derivatives can affect the functional activity of the GABA-benzodiazepine receptor-complex (GABA-BD-RC) and, in particular, by displacing diazepam from its specific binding sites [6]. The search for an endogenous modulator of GABA-BD-RC has led to the isolation and identification in brain neurons of a specific protein, diazepam-binding inhibitor (DBI), which, by its action on benzodiazepine receptors, reduces the ability of GABA to bind with specific receptors [1, 3].

In the investigation described below changes in the DBI level were studied in animals with immobilization stress and during its correction by diazepam and by N^6-cyclohexyladenosine, an agonist of A_1-adenosine receptors.

EXPERIMENTAL METHOD

Experiments were carried out on 45 noninbred male albino rats weighing 200-220 g. Immobilization stress was induced in the animals by fixing them for 6 h. The following substances were used in the experiments. N^6-cyclohexyladenosine, an agonist of A_1-adenosine receptors (from the All-Union Technologic Research Institute of Antibiotics and Enzymes of Medical Importance, Leningrad) in a dose of 0.1 mg/kg, and diazepam (from "Polfa," Poland) in a dose of 0.5 mg/kg. The substances were injected intraperitoneally 30 min before the beginning of the experiment and thereafter every 2 h during its course. Animals of the control group received physiological saline. The animals were decapitated. The brain was quickly removed (within 30-45 sec) and homogenized in 10 volumes of hot (80°C) 1 M acetic acid. The homogenate was centrifuged at 48,000*g* for 20 min (at 2-4°C). The supernatant was adjusted to pH 6 and again centrifuged, after which the supernatant was lyophilized. The DBI level

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