rise of the serum AOA leads to a fall of the LPO level. This view is supported also by the strong negative correlation between the CP/TR ratio, reflecting the serum AOA, and the quantity of MDA accumulating in it ($r = -0.72$). The results of this investigation and also those obtained by other workers [8, 10] are evidence of the important role of the CP/TR system in the regulation of serum LPO processes. On this basis the following chain of events taking place during HPO can be postulated. An increase in the partial pressure of oxygen in the blood serum leads to activation of LPO in it [2]. This process is accompanied by a change in the lipid composition of the blood serum [4, 5], which leads to further accumulation of MDA after each successive HBO session. Later, after the 5th session, activation of the serum AOS takes place. One possible cause of the activation of this system may be a high level of LPO products in the serum. Later, against the background of high AOA, LPO processes are not activated during the HBO session. It can thus be postulated that the CP/TR system participates in protection of the body against the toxic action of oxygen.

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EFFECT OF NALOXONE ON IMMOBILIZATION-INDUCED HYPOALGESIA IN RATS

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KEY WORDS: stress-induced hypoalgesia; endogenous opioid peptides; naloxone.

Neurochemical investigations have demonstrated the involvement of both opioidergic and nonopioid mechanisms in the production of stress-induced hypoalgesia, and the prevalence of one or the other depends on the nature and duration of the stressor factors and also on activity of the animal based on them [1, 4, 6, 10]. A study of hypoalgesia developing in rats during and after short-term immobilization showed that blocking opiate receptors with naloxone does not reverse changes in the latent periods (LP) of tail withdrawal in the tail-flick test [14] or of licking the paws in the hot-plate test, although it weakens changes in nociception assessed on the basis of jumping by the animals [4]. However, there are no data on the dynamics of the change in pattern of nociceptive sensation during long-term immobilization stress and on the involvement of endogenous opioidergic mechanisms in its production.

The aim of this investigation was to study LP of nociceptive responses of rats during and after immobilization for 24 h, and also to determine the effect of naloxone, a specific blocker of opiate receptors, on these periods.

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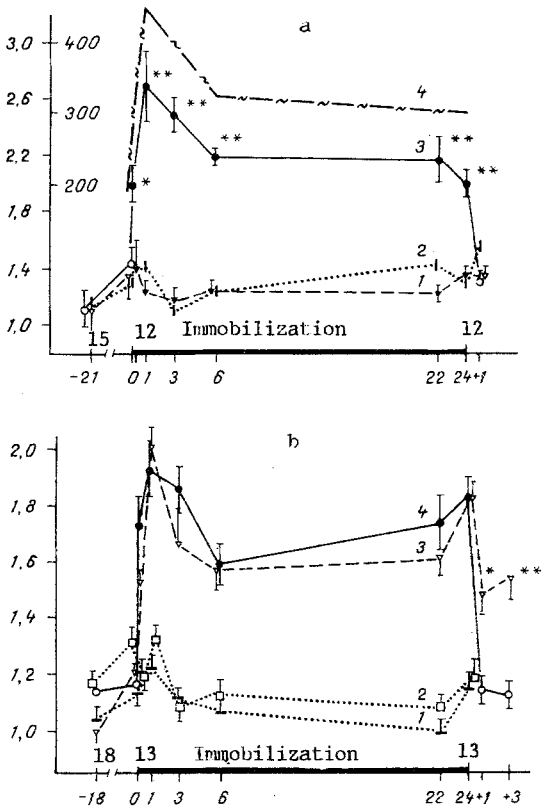

Fig. 1. Changes in LP of nociceptive responses of rats in tail-withdrawal test and during immobilization for 24 h. Abscissa: numbers above the line denote time of day, numbers below the line denote time of experiment (in h); ordinate, LP (in sec). a: 1) Deprivation of food and water, 2) control, 3) immobilization, 4) concentration of immunoreactive β-endorphin with 50% cross-reactivity to β-lipotropin (in pg/ml, ordinate on the right) in rats' blood plasma [2]. *P < 0.01 **P < 0.001 — significance of differences (Student's t test [3]) in immobilized rats compared with two other groups of animals; b: 1) naloxone, 2) physiological saline, 3) immobilization + naloxone, 4) immobilization + physiological saline. *P < 0.01, **P < 0.001 — significance of differences between LP in animals after long-term immobilization and receiving naloxone and animals of other groups.

EXPERIMENTAL METHOD

Nociceptive sensitivity was assessed by the tail withdrawal method [8], consisting of measuring LP [3] of the motor response of the rat's tail to immersion in hot water (60 ± 1°C). Two series of experiments were carried out on noninbred male rats weighing 220-250 g. In series I (33 rats) LP was determined in three equal groups of animals: control rats, kept in a communal cage with free access to water and food, experimental rats immobilized for 24 h in standard transparent plastic cages (15 × 6 × 5.5 cm), and also rats kept in a communal cage and deprived of food and water for 24 h (immobilization control). The times of testing relative to the beginning of the experiment and also the time of day at which they were done are indicated in Fig. 1a. In series II (36 rats) LP was determined in rats of four equal groups: 1) immobilization for 24 h and injection of 1 mg/kg of naloxone ("Endo Laboratories," USA); 2) immobilization for 24 h and injection of physiological saline; 3) deprivation of food and water for 24 h, unrestrained rats receiving an injection of naloxone; 4) deprivation of food and water for 24 h and injection of physiological saline. Naloxone or physiological saline was injected subcutaneously at the base of the spine in a volume of 0.1 ml/100 g body weight 15 min before each testing of LP. The testing times are indicated in Fig. 1b.

EXPERIMENTAL RESULTS

In the experiments of series I LP of the control animals during periodic testing for a long period of time was characterized by great constancy and by minimal variability within one group, and deprivation of food and water caused no statistically significant changes (Fig. 1). In immobilized animals, at all time periods studied, a significant increase was found in the duration of LP, evidence of the development of stress-induced hypoalgesia. The greatest increase in LP was observed from the 1st through the 6th hour of immobilization, with a peak after the first hour (about 200% higher than the background value). Values of LP 1 h after the end of immobilization and after return of the animals to the case with free access to food and water were the same as in the control.

According to data in the literature, both opioidergic and nonopioid mechanisms are involved in the production of hypoalgesia during immobilization stress [4, 14]. Previously, when analyzing concentrations of immunoreactive β-endorphin with 50% cross-reactivity to β-lipotropin in the blood plasma of rats immobilized for 24 h, showed a significant increase in the concentration of these opioid peptides with a peak after 1 h [2]. High correlation between changes in the levels of these substances and thresholds of nociceptive responses (Fig. 1a) suggested that these processes are closely interconnected. To test this hypothesis, in the experiments of series II, LP was estimated in immobilized rats with their opiate receptors blocked by naloxone. Naloxone is known to be a specific antagonist of endogenous opioid peptides (endorphins, enkephalins) and of the most important narcotic analgesics (heroin, morphine, codeine, etc.) and has high binding capacity with μ-, κ- and σ-types of opiate receptors [9, 12].

The results of this series of experiments showed (Fig. 1b) that blocking opiate receptors with naloxone did not cause any statistically significant changes in LP both in control rats and after different periods of immobilization.

The results are thus evidence that endogenous opioid mechanisms do not play an important role in maintenance of the normal level of sensitivity to pain and of hypoalgesia formed during long-term immobilization stress. The fact that naloxone had no effect on stress-induced hypoalgesia also was demonstrated during strong electrodermal stimulation of rat limbs [6, 13], a procedure leading to marked readjustment of endogenous opioidergic mechanisms [4, 10, 11].

In the absence of any significant differences in thresholds of nociceptive responses in immobilized rats receiving an injection of naloxone, marked changes in LP were found after the end of stress and after the animals had been returned to their original cages (Fig. 1b). Whereas in immobilized rats receiving physiological saline the thresholds of the nociceptive responses after a stay of 1 h under unrestrained conditions did not differ from the control, in rats which received naloxone (6 injections) in the course of immobilization, significant ($p < 0.001$) hypoalgesia was found both 1 h (naloxone) and 3 h (physiological saline) after its end, compared with all the other groups of animals. These changes in nociception may be connected with differences in behavior of the animals of the group under investigation, reflected in postures of attack and defense, by standing up vertically, and by aggressive contacts accompanied by vocalization. There is evidence that aggressive–defensive behavior of animals is closely interconnected with changes in nociception [6, 7, 13].

The causes of the aggressiveness and hypoalgesia discovered in animals which received frequent injections of naloxone in the course of long-term immobilization are not yet known, but it can be postulated that interaction between a long-term state of stress and repeated injections of naloxone, accompanied by significant changes in sensitivity of opiate receptors [5], is the critical factor in the realization of this phenomenon. Potentiation of hypoalgesia during electrical stimulation of the limbs was found previously in animals when naltrexone, a blocker of opiate receptors, was used before the beginning of the experiment. It is also known that blocking of opiate receptors by naloxone produces a dose-dependent increase in the "punishing" effects of electrodermal stimulation [15] and also potentiates manifestations of inert behavior and defensive boxing in animals undertaking a behavioral task with negative reinforcement [7], which in the opinion of the authors cited is connected with potentiation of the aversiveness of nociceptive stimulation through the diminution of stress-induced hypoalgesia by naloxone. However, the absence of changes in LP in animals immobilized for a long time, discovered in the present experiments in which naloxone was used, does not agree with this opinion. Everything suggests an important role of compensatory readjustment of opiate receptors when blocked in the long term or periodically by naloxone, in

behavioral changes in animals and shifts of sensitivity to pain accompanying them after the end of long-term exposure to stress.

These experiments thus showed that long-term immobilization of rats gives rise to lasting nonopioidergic hypoalgesia, which continues throughout the period of immobilization and is unconnected with hunger and thirst of the animals. After the termination of immobilization this hypoalgesia quickly disappears. The repeated use of the opiate antagonist naloxone throughout the period of immobilization leads to spontaneous aggressiveness of the animals and reliable hypoalgesia when it ends.

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COOLING THE VASCULAR BED OF AN ORGAN MAKES ANOXIA A LESS EFFECTIVE STIMULUS

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In publications devoted to the study of the effect of oxygen deficiency in the blood and of acute hypothermia on the arteries [3, 7] and veins [15] of organs and on their filtration–absorption ratios [4, 6, 10, 11, 13], no information can be found on the combined action of these two factors. The study of this problem is of great theoretical and practical importance for the characterization of the joint functions of peripheral vessels. This has become possible thanks to the development of new techniques whereby the parameters of the resistive, capacitive, and metabolic functions of the vascular bed of an organ can be recorded simultaneously [8].

The aim of this investigation was to compare the magnitude and direction of changes in the macro- and microhemodynamics in skeletal muscle and the small intestine during exposure to the separate and combined action of cooled blood and an anoxic stimulus on the vascular bed.

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