pain sensitivity following immunization of rats with AII conjugates is yet another argument in support of the view that the angiotensin and opiod systems of the body are functionally connected.

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PLASMA INTERLEUKIN-1 ACTIVITY IN DOGS DURING WORK-INDUCED HYPERTHERMIA

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KEY WORDS: interleukin-1, work-induced hyperthermia.

It has recently been suggested that pyrogen-like substances are involved in the development of work-induced hyperthermia [10]. An urgent problem is now the study of the role of interleukin-1 (IL-1), also known as endogenous pyrogen, in the regulation of the heat exchange during physical work. Data have been obtained to show that endogenous pyrogen enters the bloodstream after prolonged muscular work. Further investigations [4, 8] led to the discovery of a high blood IL-1 level in volunteers 3-6 h after work on a bicycle ergometer. According to the authors cited, this fact is evidence of de novo IL-1 synthesis during stress, caused by intensive muscular activity. The aim of the present study was to investigate activity of this cytokine in the course of physical work.

EXPERIMENTAL METHOD

Experiments were carried out on eight male mongrel dogs weighing 17-20 kg. Physical work consisted of weight bearing by the animal on its back [2]. The magnitude of the single static load was determined individually for each dog and amounted to 80% of the heaviest weight it could carry. The work was done for 1 h in a room with a temperature of 21 ± 1°C. Blood was

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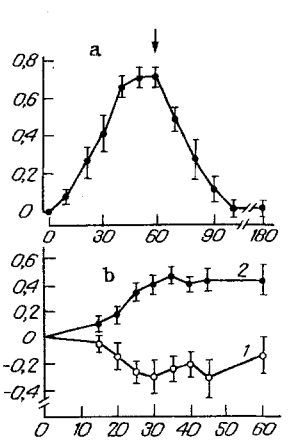

Fig. 1

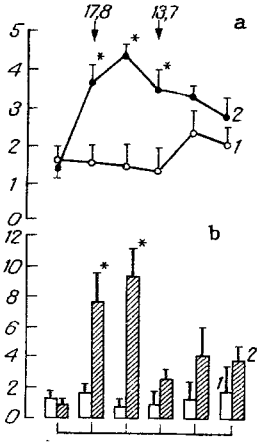

Fig. 2

Fig. 1. Changes in rectal temperature of dogs during physical exercise (a) and temperature response of C57BL/6 mice (b) to intravenous injection of 0.2 ml of dog's blood plasma obtained before (1) and after 60 min of exercise (2). Abscissa, time (in min); ordinate, temperature (in °C). Arrow indicates end of exercise.

Fig. 2. Lymphocyte-activating (a) and pyrogenic (b) activity of IL-1 in blood plasma fractions of dogs obtained before (1) and after 60 min of exercise (2). Abscissa, fractions of blood plasma (1 ml of each); ordinate: a) level of incorporation of ^{3}H-thymidine (in cpm × 10^3), b) temperature index (in cm^2). Numbers and arrows indicate region of elution of standards. Asterisk indicates significance ($p < 0.05$) of difference from level before exercise.

taken from veins of the hind limbs before, during, and immediately after work. The plasma was kept at --20°C. Samples of plasma measuring 0.5 ml were fractionated on a column (0.8 × 52 cm) with Sephadex G-50 ("Pharmacia"). The column was calibrated with standard substances of known molecular weight. Pyrogenic activity of IL-1 was determined by measuring the temperature response of C57BL/6 mice to intravenous injection of 0.2 ml of the dog's blood plasma or of fractions of eluate. The mice were kept in individual boxes in a heat chamber at 33°C. The animal's rectal temperature was measured by means of copper-contantan thermocouples and an F116/1 microvoltammeter. The temperature index was calculated as the area measured below measured below the temperature curve by means of a Leitz ASM semiautomatic analyzer, and expressed in square centimeters. Activity of IL-1 was determined in the thymocyte test [9]. Thymus cells from C3H mice in a concentration of 1 × 10^6 cells/well were incubated in medium RPMI-1640 in the presence of concanavalin A (1 μg/ml) at 37°C in an atmosphere containing 5% CO_2. The volumes of the fractions were 50% of the volume of the test cultures. ^{3}H-Thymidine (1 μCi/well, "Izotop" All-Union Combine) was added in the last 20 h of the 3-day culture. Cell suspensions were collected and deposited on a filter with the aid of an instrument of "Harvester" type. Radioactivity was determined on a β-counter (Mark III).

EXPERIMENTAL RESULTS

The experiments shows that the animal's rectal temperature rose by 0.70 ± 0.05°C after 40-50 min of physical work and remained at that level until the end of work (Fig. 1a). This indicates that a certain level of work-related hyperthermia had developed. The experiments also showed that the work done was accompanied by the appearance of endogenous pyrogen in the dogs' blood. The blood plasma obtained from the animals at the height of work hyperthermia, when injected intravenously, raised the rectal temperature of the mice (Fig. 1b).

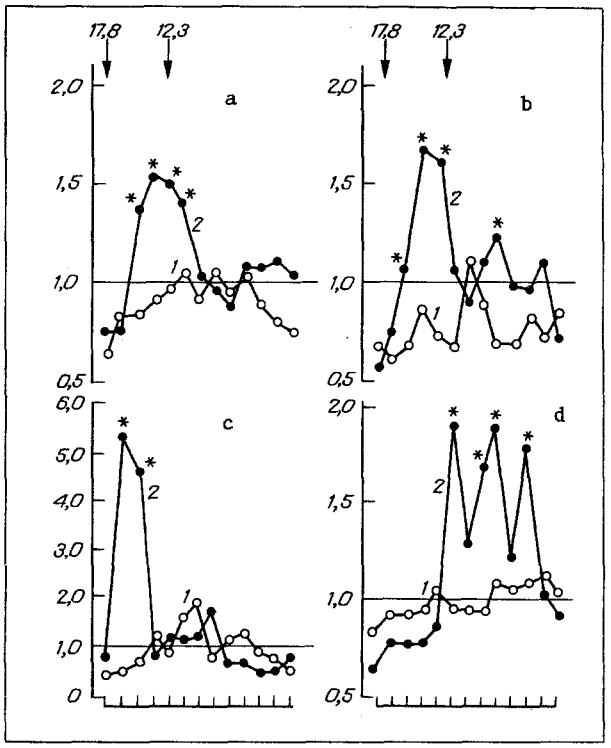

Fig. 3. Changes in IL-1 activity in fractions of blood plasma from dogs (2) after 10 (a), 20 (b), and 60 (c) min of physical work and after 3 min (d) of rest, relative to initial level (1). Abscissa, fractions of blood plasma (each 1 ml); ordinate, index of stimulation — ratio of DNA synthesis in test cultures with samples for investigation to DNA synthesis in control test cultures. Remainder of legend as to Fig. 2.

Proof has now been obtained that IL-1 and endogenous pyrogen are identical [3, 7]. The view is also held [1] that pyrogen-like substances formed during work-related hyperthermia are different from classical leukocytic pyrogens. In the present experiments, fractionation of the specimens of blood plasma showed that their pyrogenic activity is due to the action of IL-1. Fractions of eluate corresponding to the region of elution of IL-1 (12-18 kD) possessed activity in the thymocyte test and also pyrogenic activity (Fig. 2). In the resting state, pyrogenic and lymphocyte-activating activity of IL-1 were absent in the blood of normal, nonfebrile animals.

In view of the suggestion that IL-1 is involved in the mechanisms of work-related hyperthermia, we studied activity of this cytokine in blood plasma fractions from dogs during exercise. The lymphocyte-activating activity of IL-1, incidentally, was found at different times after the beginning of physical work (Fig. 3a, c). However, its high activity did not always last throughout the period of work. It will be evident that different mechanisms exist for controlling the blood IL-1 activity during physical work. One such mechanism may be proteolytic destruction of IL-1. In our experiments high activity in the thymocyte test was possessed by fractions of dog blood plasma with mol. wt. of 4-6 kD, obtained after 3-7 min of rest (Fig. 3d). It was shown previously [6] that a factor with mol. wt. of 4.2 kD, causing proteolysis of muscle tissue, and also raising the temperature of mice, but not of rabbits, is part of the IL-1 molecule, degraded by trypsin. According to some data [4], after cessation of physical work the lymphocyte-activating activity of IL-1 in the blood plasma of volunteers did not differ significantly from its level before work. With the ending of work, and during normalization of the rectal temperature in dogs, the IL-1 circulating in the bloodstream evidently undergoes proteolysis.

The results are evidence that IL-1 is secreted into the bloodstream during intensive muscular activity at very short times after the beginning of work, suggesting the existence of a reserve pool of this biologically active substance. The problem of the actual sources of IL-1 production and the mechanisms regulating its level in the blood during physical work requires further study.

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EFFECT OF CYCLOHEXIMIDE BLOCKADE OF PROTEIN SYNTHESIS IN RATS ON ALCOHOL MOTIVATION

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KEY WORDS: alcohol motivation, behavior of rats, peptides, cycloheximide.

It has been shown that cycloheximide, which blocks ribosomal protein synthesis [2], when injected into the lateral ventricles, depresses food-motivated and defensive behavior and self-stimulation in rabbits [1, 5, 6]. Against the background of the action of cycloheximide, food-motivated behavior is restored by administration of pentagastrin [5], self-stimulation by $ACTH_{4-10}$ [1], and defensive behavior by bradykinin [6]. These findings are evidence that biological motivations are realized as the corresponding behavior as a result of expression of special protein molecules by the genome of brain neurons.

Alcohol motivations are formed in animals on the basis of biological motivations of fear, thirst, etc., when artificially replaced by the taking of ethanol [4]. It can be tentatively suggested that realization of the taking of alcohol by animals with artificially formed alcohol motivation also is determined by expression of specific protein molecules by the genome of brain neurons.

To solve this problem, in the investigation described below alcohol motivation, formed artificially in rats on the basis of water deprivation, was studied during administration of cycloheximide, a blocker of protein synthesis. In view of data in the literature pointing to the initiating role of the perifornical region of the hypothalamus in the mechanisms of alcohol motivation, formed on the basis of water deprivation in animals [3], the investigative behavior of rats and their ethanol consumption also were investigated during blockade of protein synthesis by cycloheximide, injected directly into this region.

EXPERIMENTAL METHOD

Experiments were carried out on 31 noninbred rats (males weighing 200-300 g). Alcohol motivations were formed in the rats by providing them with a 20% solution of ethanol for 4-5 months as the sole source of fluid. The rats were then transferred to individual cages and the daily consumption of 20% ethanol solution, food, and water was recorded. Animals with a formed

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