PHYSIOLOGICAL RESPONSE IN RATS TO PROTEIN CONJUGATES OF ANGIOTENSIN II DURING LONG-TERM IMMUNIZATION

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The role of angiotensin II (AII) in the regulation of physiological functions has been the subject of much careful study. The role of AII in the regulation of the hemodynamics, the renal blood flow, drinking and alcohol-directed motivation, certain forms of goal-directed behavior, and the release of other physiologically active substances has been distinguished [5, 11, 15]. Nevertheless, the forms and level of interaction of AII with other components of neurohumoral regulation, which could explain the phenomenon of polyfunctionality of this peptide, remain inadequately lucidated.

Attempts were made previously to use immunization with conjugates of AII to correct an experimental hypertensive state. However, in studies conducted on various models of stable hypertension (renal, doca—salt, hereditarily determined) it was not always possible to obtain an effective reduction of the blood pressure, despite the fact that antibodies against AII were found in the blood of animals immunized with this peptide [9, 10]. It has become evident that the use of immunization by peptides as a method of goal-directed modification of the physiological state of an organism [1] demands a wider selection of behavioral and biochemical parameters to be recorded. It was shown previously that a 4-month cycle of immunization of rats with protein conjugates of AII leads not only to the appearance of antibodies against this peptide, but also to an increase in the activity of AII-generating peptidase (angiotensin-converting enzyme) and to elevation of the AI level in the blood [4].

The aim of this investigation was to study changes in a number of physiological parameters and also in the response to immobilization stress during long-term immunization of rats with protein conjugates of AII.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 200-250 g, divided into four groups with 10 rats in each group. Group 1 consisted of rats immunized with a conjugate of AII with bovine serum albumin (BSA) together with Freund's complete adjuvant, group 2 of rats receiving injections of AII, group 3 of rats immunized with BSA (with Freund's adjuvant), and group 4 of rats receiving injections of physiological saline. All the animals received these substances in accordance with a chosen plan of immunization: during the first month weekly injections, followed by maintenance injections at monthly intervals [9]. Throughout the experiment, which lasted 13 months, there were two pauses (2 and 3 months) (Fig. 1). The conjugate was injected subcutaneously into the upper thirds of the rats' limbs in a volume of 0.1 ml per animal. The AII-amide conjugate (Experimental Factory, Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR) with BSA ("Serva") was synthesized by the carbodiimide method in accordance with [13].

Blood for immunochemical investigation was obtained from the subclavian vein. The titer of antisera to AII and BSA was determined by enzyme immunoassay using polystyrene planchets [4].

During the long-term experiment the following physiological parameters were determined regularly for all the rats: 24-hourly consumption of food and water, level of motor activity using the "Varimax" apparatus (Columbus Instrument, USA),

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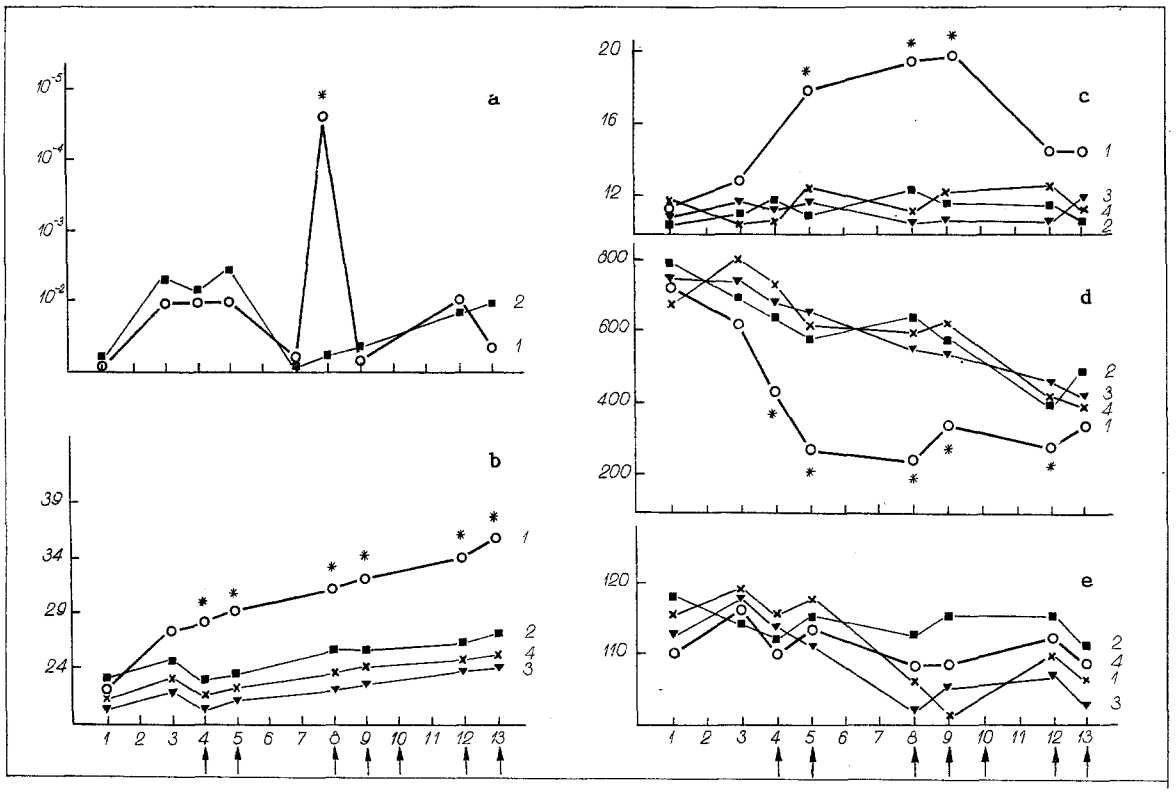

Fig. 1. Changes in antibody titer and physiological parameters in rats immunized with protein conjugates of AII. a) Antibody titer against AII, b) 24-hourly water consumption (in ml), c) threshold of pain sensitivity (in sec), d) motor activity (number of movements performed by animals during 5 min), e) blood pressure (in mm Hg). Abscissa, time of experiment (in months); ordinate, antibody titer and physiological parameters. Arrows indicate times of booster immunizations. Asterisks indicate significant changes in experimental group compared with animals of control group. 1-4) Groups of animals.

the level of pain sensitivity by the tail withdrawal test using temperature stimulation, and the systolic blood pressure by an indirect method in the caudal artery.

The animals were kept on a standard diet with a normal salt content, and with free access to food and water.

The results were subjected to statistical analysis by Student's test and by the nonparametric signs test.

EXPERIMENTAL RESULTS

Immunization of the 2-month-old rats with a conjugate of AII and BSA led to gradual elevation of the antibody titer in most animals. By the end of the 3rd month of immunization the titer of antiserum against AII, found in eight of the 10 rats of group 1, averaged 1:175. After a pause of 3 months, immunization led to a sharp rise of the antibody titer to AII in all animals of this group. The average titer was 1:47,000, varying in different individuals between 1:320 and 1:160,000. Subsequent injections of the conjugate of AII with BSA, given at monthly intervals or after a longer pause (Fig. 1a) did not cause any significant increase in the titer of antibodies to AII.

The data in Fig. 1 also show the titer of angiotensin antibodies following injection of AII alone into the rats (group 2). Fluctuations of the titer between 1:80 and 1:320 were observed in these animals during the first 3 months of immunization. No significant increase in the titer was observed to injection of AII. In groups 3 and 4 respectively no antibodies to AII were found.

The results thus indicate the existence of a definite age dynamics of the immune response to injection of the protein conjugate of AII. The peak of activity of antibody formation after regular monthly injections of the conjugate followed by a pause of 3 months corresponds to rats aged 7-8 months. Later injection of the conjugate of AII with BSA no longer caused any further increase in the immune response.

TABLE 1. Changes in Threshold of Pain Sensation (in sec, $M \pm m$) in Rats Immunized with AII Conjugates before and after Immobilization Stress

Group of rats	Before immobilization	30 min after immobilization	2 h after immobilization	24 h after immobilization
1 (n=10)	18,5±1,9**	26,5±1,3*	24,1±1,5*	22,2±2,1*
2 (n=8)	12,8±1,6	16,9±1,2*	15,5±1,3*	13,2±1,7
3 (n=6)	13,2±1,4	17,8±1,4*	16,5±1,1*	12,4±2,2
4 (n=6)	11,1±2,2	15,8±1,6*	14,7±1,4	10,8±2,4

Legend. *$p < 0.05$ compared with initial level; **$p < 0.05$ compared with groups 2, 3, and 4 before immobilization.

During immunization definite and prolonged changes in a number of parameters were found in the animals of group 1. The water consumption of the rats increased successively, and exceeded the initial level on average by 74% by the end of the experiment. In the remaining groups these changes were nothing more than a tendency without statistical significance (Fig. 1b).

A considerable decrease in motor activity and, conversely, a rise of the threshold of pain sensation were observed in this same group of rats (Fig. 1c, d). As the titer of antibodies to AII fell, the parameters of motor activity and the threshold of pain sensation in group 1 returned close to the initial values and to those in the control.

The systolic blood pressure showed a tendency to fall during immunization, and this was particularly marked in group 1 in the period immediately after the peak of the immune response. However, these changes did not differ so significantly from those observed in the control groups and returned to their initial level by the end of the experiment (Fig. 1e).

Table 1 gives the results of determination of the threshold of pain sensation in animals subjected to short-term immobilization stress. Immobilization for 30 min (fixation of the rats in the supine position on a flat board) led to a significant rise in the threshold of response to nociceptive stimulation in all groups of animals. However, since initially (before immobilization, at the peak of antibody formation) the threshold of pain sensitivity in the rats of group 1 was significantly higher, the the absolute value of this parameter after exposure to stress was higher than in control groups. This means that rats immunized with the AII conjugate were absolutely more resistant to pain, when tested under conditions of immobilization stress. Reduced reactivity to pain continued in the animals of group 1 on the 2nd day, whereas in the remaining animals the levels of the threshold returned to their initial values in the course of 2-4 h after the end of immobilization.

Active immunization of animals with conjugates of regulatory peptides was tested previously in investigations with bradykinin [2], β-endorphin [3, 6, 14], luliberin (LHRH) [7], and AII [9, 10]. The essence of this approach is that the specific antibodies formed during immunization with the peptide depress its endogenous concentration, thereby changing the level of regulatory activity in the body. Immunization with β-endorphin led, in particular, to significant changes in behavioral activity of animals belonging to different evolutionary groups [3].

However, examination of the results obtained by this approach reveals several essential and general factors. First, just as in previous studies [2, 8], the present investigation shows that changes in physiological parameters agree as a whole with a rise of the antibody titer to AII and, correspondingly, its return to the original level as the immune response diminishes. The maximal inunune response to injection of the conjugate of AII with BSA was observed in animals aged 7-8 months, and was much weaker thereafter.

Second, long-term immunization may lead not to a decrease, but on the contrary, to an increase in the concentration of the endogenous peptide against which the immunization had been carried out. The present investigation, in particular, showed an increase in water consumption in rats immunized with the AII conjugate, and this continued to increase even after the end of the immune response to AII.

Third, measurement of a wider series of physiological parameters would reveal that AII is probably involved in the regulation of the general motor activity of animals and their sensitivity to heat-related nociceptive stimulation. These results confirm reports of behavioral reactions connected with central and peripheral injections of angiotensin, such as the passive avoidance reaction [8], and the threshold of convulsive reactions to injection of neuroleptics [12]. Changes in the threshold of

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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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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