TRENDS OF NOCICEPTIVE SENSITIVITY AND PLASMA STEROID HORMONE LEVELS IN WISTAR RATS

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UDC 612.884-08:[612:128:[577.175.53+
577.175.624

KEY WORDS: nociceptive sensitivity; corticosterone; testosterone

The discovery of factors determining genetic and individual resistance of the organism during the development of emotional stress is an urgent task in contemporary physiology and medicine [3]. In negative emotional states nociceptive sensitivity has been shown to be changed [1, 5]. A widely used method of determining nociceptive sensitivity is to record the latent period (LP) of the response of an experimental animal to a graded nociceptive stimulus. It can be postulated that repeated exposure to a nociceptive stimulus in the course of testing will induce changes in nociceptive sensitivity, which can be used to characterize the experimental animal's individual properties.

The aim of this investigation was to study the dynamics of nociceptive sensitivity in Wistar rats during repeated exposure to a standard nociceptive stimulus. The animal's physiological state was assessed on the basis of plasma levels of steroid hormones: corticosterone (CS) and testosterone (TS).

EXPERIMENTAL METHOD

Experiments were carried out on 226 male Wistar rats weighing 150-200 g. The nociceptive sensitivity of each animal was determined by measuring LP of the tail flick response (TFR) by means of the "Tail Flick Unit" (Ugo Basile, Italy). The rat was placed in a Plexiglas constraining cage, limiting its movement. After immobilization for 10 min the top part of the tail was exposed to infrared radiation at a temperature of 53°C. The time from switching on the infrared lamp to tail flicking was recorded by an electronic timer. LP for each rat was measured 10 times with intervals of 60 sec between exposures.

To determine plasma levels of steroid hormones the tip of the tail was removed, after which microsamples of blood (0.02 ml) were taken at certain stages of the experiment: sample 1 during 20-40 sec after placing the rat in the cage, sample 2 after immobilization of the animal for 10 min, sample 3 after nociceptive stimulation of the immobilized rat.

Plasma CS and TS concentration were determined by radioimmunoassay.

The results were subjected to statistical analysis by computer, using dispersion analysis (Fisher's test) and Student's t test.

EXPERIMENTAL RESULTS

Since the procedure of amputation of the tip of the tail can affect the rat's nociceptive sensitivity, the time course of changes in LP of TFR during repeated stimulation was first investigated without monitoring the plasma CS and TS levels.

The trend of change of nociceptive sensitivity during 10 applications of the stimuli was studied in 91 animals.

Analysis of the experimental results led to subdivision of the experimental animals into six groups. Group 1 consisted of 18 rats with high nociceptive sensitivity (LP 3-3.5 sec), which was maintained throughout the period of recording with only insignificant scatter of the LP values for individual animals (Fig. 1a). Group 2 consisted of 19 rats with the low

P. K. Anokhin Research Institute of Normal Physiology, Academy of Medical Sciences of the USSR, Moscow (Presented by Academician of the Academy of Medical Sciences of the USSR K. N. Sudakov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 105, No. 5, pp. 526-529, May, 1988. Original article submitted January 12, 1987.

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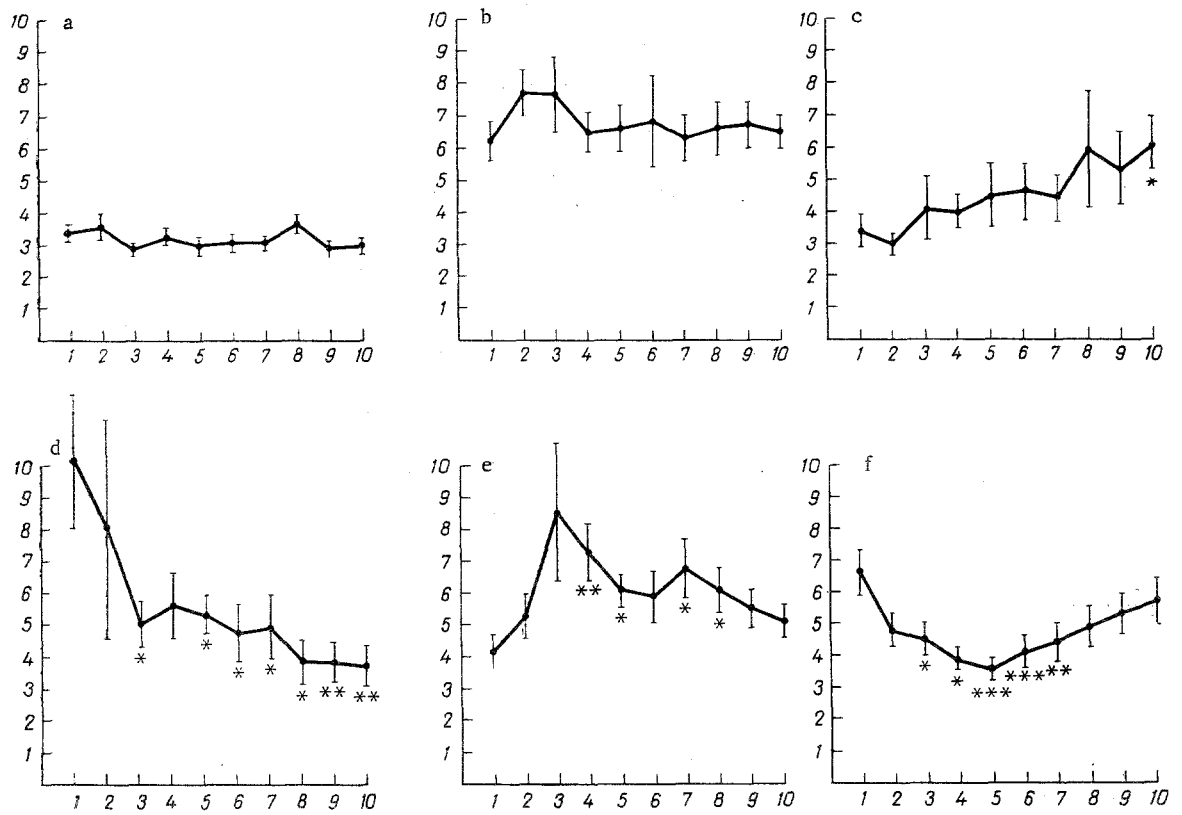

Fig. 1. Time course of nociceptive sensitivity in Wistar rats. Abscissa, time (in min); ordinate, LP of TFR (in sec); n) number of animals. *p < 0.05, **p < 0.01, ***p < 0.001 for comparison of mean values of LP when tested after 1 min with mean values for all subsequent times of determination. Explanation in text.

nociceptive sensitivity. LP of their TFR varied from 6 to 8 sec, with considerable deviations of individual values in some animals (Fig. 1b).

Among Wistar rats it is thus possible to distinguish groups with high and low sensitivity to nociceptive stimulation, persisting throughout the investigation. However, in most rats during repetitive nociceptive stimulation changes were observed in LP of TFR. For instance, the 11 rats of group 3 were distinguished initially by low values of LP in the last minutes of recording (Fig. 1c). In the animals of this group nociceptive sensitivity decreased with an increase in the number of stimuli presented. Rats of group 4 (9 animals) were characterized by initially high values of LP, which gradually decreased toward the end of testing (Fig. 1d). In this case nociceptive sensitivity increased.

Animals placed in groups 3 and 4 thus were distinguished by opposite changes in nociceptive sensitivity. In the first case it decreased, in the second it increased.

Rats in which changes in LP corresponding to hyper- or hypoalgesic states discovered during testing reached the initial values in the last minutes of recording were thus distinguished. For instance, among 16 rats of group 5, during the first minutes of the test low values of LP were observed, but they increased significantly at the 4th, 5th, 6th, 7th, and 8th minutes of recording, and then decreased again to the original level (Fig. 1e). The nociceptive sensitivity of rats of this group decreased during testing and reached the initial value on the last presentations of the stimulus. Finally, in the 17 rats of group 6 long LP in the first minutes of recording began to fall after 3 min, to reach minimal values by the 5th minute, after which they gradually increased to the original level (Fig. 1f). Thus hyperalgesia discovered during the investigation was replaced by a period of low nociceptive sensitivity. Only one rat of the population which we tested was distinguished by extremely high values of LP, up to 100 sec, in any of the above-mentioned groups.

To monitor the physiological state of the animals, plasma levels of steroid hormones were determined in 96 rats. Analysis of the results showed that immobilization for 10 min caused the CS level to rise from 14.6 to 23.5 μg% ($p < 0.001$), whereas the TS concentration

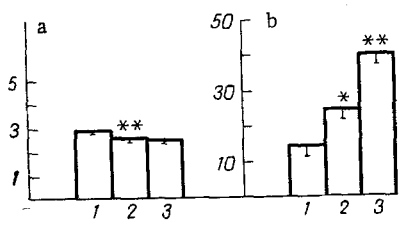

Fig. 2

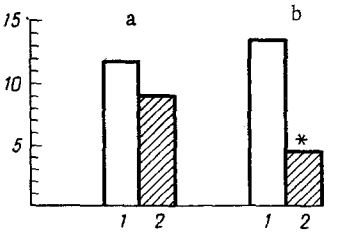

Fig. 3

Fig. 2. Effect of immobilization and nociceptive stimulation on plasma steroid hormone level of rats. Ordinate: a) TS concentration (in ng/ml; 76 rats); b) CS concentration (in μg%; 96 rats). 1) Intact animals; 2) after immobilization for 10 min; 3) after nociceptive stimulation of immobilized animal for 10 min. *$p < 0.05$, **$p < 0.001$.

Fig. 3. Elevation of plasma CS level (in μg%) of rats after immobilization and nociceptive stimulation. 1) Experimental group (100 rats) after immobilization for 10 min (a) and nociceptive stimulation during immobilization (b); 2) control (35 rats) after immobilization for 10 min (a) and after subsequent immobilization for 10 min without nociceptive stimulation (b). *$p < 0.01$.

fell at the same time from 2.97 to 2.57 ng/ml ($p < 0.001$). After tenfold nociceptive stimulation of the immobilized animals, the TS concentration remained low, but the CS level rose once again from 23.5 to 40.7 μg% compared with the postimmobilization period ($p < 0.001$; Fig. 2). Plasma CS concentrations were monitored in 35 rats. Their CS concentration was determined after immobilization for 10 and 20 min. Comparison of the increase in the CS concentration in rats of the control and experimental groups showed that the postimmobilization CS level was significantly increased after nociceptive stimulation and unchanged in the absence of such stimulation (Fig. 3).

It can be concluded from these experiments that both immobilization for 10 min and nociceptive stimulation are factors of negative emotional significance for the experimental animals, causing significant changes in the plasma CS and TS levels. Tenfold nociceptive stimulation was carried out against a hormonal background which had already been changed by immobilization, and it induced a further increase in the plasma CS concentration, whereas no difference was found in the increase in CS after immobilization for 10 min and for 20 min. Consequently, nociceptive stimulation considerably potentiates the negative emotiogenic action of immobilization.

It can be tentatively suggested that the differences revealed in the time course of nociceptive sensitivity in rats of the same strain are determined by their individual differences and, perhaps, by the zoosocial behavior of the animals.

The results confirm the individual differences discovered previously in the response of experimental animals to factors forming a negative emotional state [3].

Since the development of prognostic criteria for predicting the response of an individual to a conflict situation is of great importance at the present time, and considering that hormonal-peptide-neurochemical mechanisms lying at the basis of formation of negative emotional states determine the characteristics of nociceptive sensitivity [2, 4], the differences revealed by this investigation in the time course of nociceptive sensitivity can evidently be used as a prognostic criterion for predicting the possible response of an animal to an unfavorable situation.

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EFFECTOR MODELLING OF THE ACTION OF GABA-RECEPTOR-COMPLEX LIGANDS. FUNCTIONAL INTERACTION BETWEEN SUBUNITS OF THE COMPLEX

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UDC 612.822.014.467:[615.21:547.466.3

KEY WORDS: GABA-receptor complex; exogenous ligands; pharmacological effect; cooperativeness of interaction

The study of the structure and function of the GABA-receptor complex (GABA-RC), the postsynaptic supramolecular receptor-channel assembly, including receptors of GABA, bicuculline (BC), benzodiazepines (BD), their reciprocal agonists and antagonists, barbiturates (BB), and picrotoxin (PT), has mainly been undertaken by radioligand methods in vitro [6, 12-14]. The principles of function of GABA-RC in vivo can be determined by studying the time course of the rapidly reversible pharmacological effects of exogenous ligands by the use of techniques ensuring mutually equal agreement between state of the biological systems and the influences to which it is exposed [8, 10]. These demands are satisfied by investigations of changes in minimal effective doses and the shape of the distribution of probability of the recorded effects during infusion of convulsive agents which are GABA-RC ligands against the background of modulating influences of BB and BD.

Radioligand studies [7, 12-14] and the use of photoaffinity labeling of BD-receptors [12, 15] have shown that GABA-RC incorporates four BD binding sites. The ratio between the number of binding sites of BD and GABA is 1:2 [12, 13]. As a result, the hypothesis of the "quartet" model of GABA-RC, represented by four subunits, incorporating one BD binding site and two GABA binding sites, was confirmed [7, 13]. It is suggested that reception of the ligand by one of the four subunits leads to modification of the state of the whole supramolecular complex [13].

To study modifications of the state of GABA-RC under physiological conditions and to establish the parameters of these processes determined at the whole organism level, in the investigation described below the character of the changes in parameters of the convulsant effect of BC, PT, and metrazol (MT), namely the distribution of minimal effective doses, and of thiosemicarbazide (TS), namely the distribution of probability of a recordable effect following administration of BB (barbital sodium) and BD (phenazepan,* 1,2,4,5-tetrahydrophenazepam), were investigated.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA mice weighing 18-22 g. Animals in the control group and animals 1 h after intraperitoneal injection of phenazepam (0.08-5.6 mg/kg), 1,2,4,5-tetrahydrophenazepam (1.4-22.5 mg/kg), and barbital sodium (20-160 mg/kg), received BC, PT, and MT in the form of 0.1, 0.3, and 1% solutions respectively, by intravenous infusion (into the caudal vein) at the rate of 0.01 ml/sec. Minimal effective doses inducing clonicotonic convulsions (CTD) and tonic extension (TED) were determined [2-4]. The probability of development of clonic convulsions (CC) and tonic extension (TE) was determined after injection of 5-400 mg/kg TS into control mice after preliminary (30 min earlier) injection of phenazepam (0.35-2.8 mg/kg) and barbital sodium (25-200 mg/kg). The results were analyzed by algorithms described in [5, 9].

*7-bromo-1,3-dihydro-5-(2'-chlorophenyl)-2H-1,4-benzodiazepin-2-one.

Department of Physicochemical Pharmacology, A. V. Bogatskii Physicochemical Institute, Academy of Sciences of the Ukrainian SSR, Odessa. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 105, No. 5, pp. 529-531, May, 1988. Original article submitted March 10, 1987.

0007-4888/88/0005-0631$12.50