BIPHASIC SELECTIVE EFFECT OF ANTISERUM TO β-ENDORPHIN ON NOCICEPTIVE SENSITIVITY IN RATS

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Injection of antiserum to β-endorphin is used to study the role of β-endorphin in various functions of the body [4] and, in particular, in the regulation of sensitivity to pain [5]. Recent investigations, however, have been conducted only for a period of a few hours after injection of the antiserum; under those circumstances changes in nociceptive sensitivity have been studied only in response to a thermal stimulus. Yet we know that peptides, including opioid, have a more or less selective action on nociceptive sensitivity depending on the type of nociceptive stimulus [6, 9]. The aim of the investigation described below was accordingly to study the longterm effects of antiserum to β-endorphin on nociceptive sensitivity in rats during the action of nociceptive stimuli of different kinds: electrodermal and thermal.

EXPERIMENTAL METHOD

Experiments were carried out on three groups of Wistar rats weighing 180-250 g, kept when not in use in the experiments under unrestrained conditions at room temperature and with free access to water and food. During the experiment the rats were placed in a plexiglas chamber, restricting the animal's movements, and with the tail exteriorized.

In the rats of group 1 (15 animals) the threshold of the defensive tailflick response (TFR) to electrodermal (50 Hz, sinusoidal current, 2-3 sec) nociceptive stimulation (ENS), of increasing voltage, applied to the animal's tail through the wire lattice floor of the chamber, was recorded 3 times. In the rats of group 2 (50 animals) the latent period (LP) of the same TFR in response to a thermal nociceptive stimulus (TNS), applied 10 times with intervals of 2-3 min, was recorded automatically on a special apparatus (Shinghava Ltd.).

In the rats of group 3 (eight animals) thresholds of TFR in response to ENS and LP of TFR in response to TNS were recorded in each experiment.

Antiserum to β-endorphin, obtained in a chinchilla rabbit, with antibody titer of 1: 15,000-20,000, and having less than 10% cross-reactivity to δ- and κ-agonists of opiate receptors (A. B. Poletaev, P. K. Anokhin Research Institute of Normal Physiology, Academy of Medical Sciences of the USSR), was injected in a single dose into the caudal vein of the rats in a dose of 4 mg/ml (10 mg/kg body weight) in a volume of 0.5-0.8 ml. Nonimmune serum obtained from the same rabbit before immunization, or isotonic solution in the same volume, was injected as the control. Some animals also were given an intravenous injection of naloxone (Endo) in a dose of 0.15 mg/kg. The experimental results were subjected to statistical analysis by Student's paired t test.

EXPERIMENTAL RESULTS

A single injection of antiserum into the rats of group 1 (10 animals) caused gradual lowering of the threshold of TFR from 17.1 ± 1.6 V to 9.6 ± 2.7 V after only 15-20 min, and to 5.8 ± 1.8 V after 60 min, followed by gradual elevation of the threshold to 6.6 ± 1.8 V after 4 h and to 8.1 ± 1.8 V the next day (Fig. 1). During the next 3-6 days after the injection

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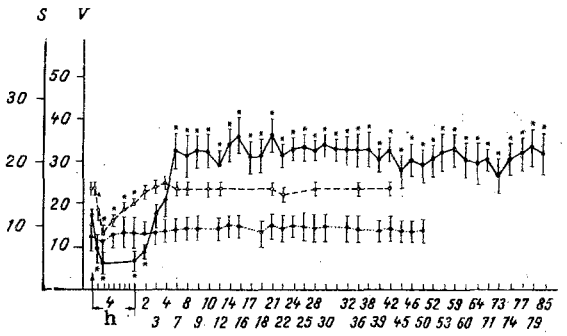

Fig. 1. Time course of changes in thresholds of TFR to ENS after injection (arrow) and antiserum (continuous line) and of nonimmune serum (dotted line) in rats of group 1 and LP of TFR to TNS after injection of antiserum (broken line) in rats of group 2. Abscissa, time (S, in sec) and thresholds (V) of voltage (in V) of TFR; ordinate, time after injection during first 4 h (arrows) and on subsequent days of observation, asterisk indicates significance of differences from background values.

values of the threshold of TFR did not differ significantly from the initial values. On the 7th day after injection, a significant rise of the threshold of TFR on average to 32.2 ± 4.2 V took place in all the experimental animals of this group, but in different animals it varied between 25 and 60 V, i.e., by between 1.5 and 3 times higher than initially. In the course of observations on this group of animals lasting 85 days, a high average threshold of TFR was maintained (Fig. 1), although on some days in all rats the threshold fell, sometimes to the background values: this was observed more often between 80 and 90 days after injection of the antiserum (Fig. 1; Fig. 2). Injection of the nonimmune serum (five animals) caused a small but not significant lowering of the threshold of TFR immediately after the injection from 12.6 ± 3.0 to 10.0 ± 3.2 V (Fig. 1), returning after only 20 min to the initial values. On the next experimental days, for 50 days of observation, fluctuations of the threshold of TFR in these rats did not differ significantly from the background values (Fig. 1).

In the rats of group 2 injection of antiserum (23 animals) caused a significant reduction of LP of TFR to TNS from 15.7 ± 0.7 to 9.0 ± 0.9 sec from the first hour after injection (Fig. 1), followed by a gradual rise during the next 4 h, but without reaching the background level. In the next 2-40 days of observation values of LP of TFR did not differ significantly from the background values (Fig. 1). Injection of nonimmune serum (27 animals) and also of isotonic solution did not cause any significant changes in this parameter of nociceptive sensitivity.

In the rats of group 3, injection of antiserum (five animals) caused a significant decrease in the threshold of TFR to ENS in the first 20 min after injection from 21.4 ± 2.1 to 13.0 ± 4.1 V, and after 1-4 h to 7.2 ± 3.2 V (Fig. 2). On the 2nd day the threshold of TFR in these animals was significantly lower than the background values, and it averaged 14.6 ± 2.0 V. In the same animals LP of TFR in response to TNS was significantly reduced during 15-60 min after injection of the antiserum from 14.2 ± 1.2 to 9.1 ± 1.1 sec, but after 3 h it was back to 14.0 ± 0.4 sec. On the 2nd day LP of TFR to TNS was 14.5 ± 0.9 sec and did not differ significantly from the background values. The threshold of TFR to ENS 7-8 days after injection of the antiserum was increased on average in all the animals to 42.4 ± 4.0 V ($p < 0.001$), i.e., was approximately doubled, but in individual animals on certain days, it was increased up to 60 V, and this rise of the thresholds of TFR was recorded for 84 days with some fluctuations of values between 2 and 8 V (Fig. 2). It was only on the 84th day that the average threshold of TFR for all the animals fell to 31.0 ± 2.0 V, although this differed significantly from the background values ($p < 0.05$). Meanwhile no significant changes were observed in LP of TFR to TNS (Fig. 2) in the same animals during 42 days of observation. Injection of nonimmune serum into three rats of group 3 caused no significant changes in the threshold of TFR to ENS or in LP of TFR to TNS in all the experimental animals.

Some rats of experimental group 3 received an injection of naloxone on various days after injection of the antiserum. Injection of naloxone on the 8th day led, after 20 min,

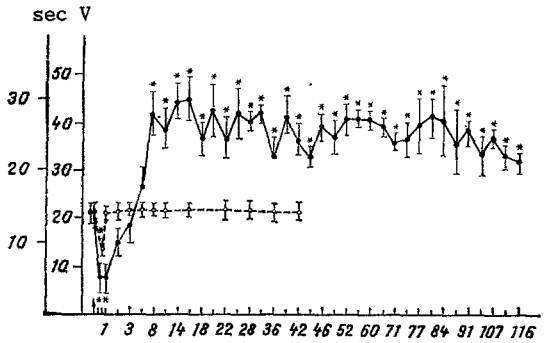

Fig. 2. Time course of changes in thresholds of TFR to ENS (continuous line) and LP of TFR to TNS (broken line) in rats of group 3 after injection of antiserum (arrow). Legend as to Fig. 1.

to lowering of the threshold of TFR to ENS from 52.0 ± 2.0 to 8.0 ± 0.1 V, after 40 min to 3.0 ± 0 V ($p < 0.001$), after 1 h to 3.5 ± 0.5 V ($p < 0.001$), and this was followed by an increase to 8.0 ± 0 V ($p < 0.001$) 2 h, and restoration of the threshold of TFR 3-4 h after the injection. A similar injection of naloxone did not cause any change in LP of TFR to TNS in the same animal (15.3 ± 0.7 sec before and 15.2 ± 0.4 sec after the injection). Injection of naloxone into two rats on the 10th day after injection of the antiserum lowered the threshold of TFR to ENS from 39 ± 2 to 15.5 ± 0.7 sec, followed by recovery after 3-3.5 h. Injection of naloxone on the 74th day also caused the threshold of TFR to fall from 35.0 ± 1 to 10.3 ± 0.3 V in one animal; in a second animal, in which an independent fall of the threshold of TFR to ENS to 14 ± 0.1 V had already been observed at this time, injection of naloxone lowered the threshold but only to 10 ± 1.0 V (difference not significant). In these same rats, injection of naloxone on the 10th and 74th days caused no change in LP of TFR to TNS.

The experiments described above thus showed that injection of antiserum to β-endorphin, at least in the titer used and in the corresponding phase, induces a biphasic effect: a short-term (up to 2 days) hyperalgesic, and a long-term (over 3 months) selective hypoalgesic or analgesic effect. In the first phase potentiation of nociceptive sensitivity was observed both to TNS, in agreement with data obtained by other workers [5], and also to ENS in the same localization, which is connected with blockade of secretion of endogenous β-endorphin as a result of the direct action of the antiserum [5]. The second phase of lowering of nociceptive sensitivity evidently reflects a unique rebound effect in response to a fall in the concentration of endogenous β-endorphin in the body, which was described in response to injection of antisera to other peptides also [2], and is manifested as activation of the endogenous antinociceptive opioid system, evidence in support of which is given by the blocking effect of injection of naloxone, which is known to be manifested only during activation of the endogenous opioid system [7].

However, this activation of the opioid system reveals a selective antinociceptive effect on ENS, but not on TNS (the same selectivity was exhibited, although to a lesser degree, in the first hyperalgesic phase also), in the same way as κ-agonists have a strong analgesic effect on pain evoked by mechanical stimulation, compared with thermal stimulation [8], whereas with δ-agonists the converse is the case [3]. The long duration of the second phase of action of the antiserum was probably connected with the fact that "action at the level of one regulatory peptide may modify the state of the whole peptide continuum, and it may do so, moreover, for a much longer time than the period of the primary direct effects of that regulatory peptide" [1].

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ACTIVITY OF POSTURAL ASYMMETRY FACTORS IN SYMMETRICAL REGIONS OF THE RAT SPINAL CORD

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Interest in the problem of chemical asymmetry of the brain continues to increase in connection with the study of functional asymmetries which are characteristic of the normal and injured CNS [10]. Comparatively recently a new class of endogenous oligopeptide factors inducing an asymmetrical distribution of tone of the limb muscles in spinalized and intact animals, leading to the formation of postural asymmetry (PA), has been discovered. Postural asymmetry factors (PAF) were first found in association with unilateral injuries of central motor systems [2, 3, 7]. Later they were found in the cerebral hemispheres [1, 8] and also in tissues of the spinal cord [9] and pituitary gland [11] of intact animals. On the basis of these data it was suggested that there exist neuropeptide modulators of a system regulating the state of muscle tone of the limbs under normal conditions and in association with unilateral injury to the CNS [4].

This paper describes a study of the distribution of PAF activity in symmetrical regions of the spinal cord of the intact animal, and also of animals with unilateral injury to the motor area of the neocortex.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 180-200 g were used. Under ether anesthesia the representation of the right hind limb in the motor area of the neocortex of the left hemisphere was removed. The animals were decapitated 48 h later, the lumbar enlargement of the spinal cord was removed and divided into right and left halves, taking bearings from the median sulci of the dorsal and ventral surfaces. The material was extracted with 0.2 N HCl by the method described previously [6]. The lyophilized extract was dissolved in 0.1% TFA and fractionated by gel-filtration on a column measuring 5 ml (0.6 × 20 cm), filled with Sephadex G-25 (superfine), in 0.1% TFA. The eluate was divided into two fractions: high-molecular-weight (over kD, V_o) and low-molecular-weight (under 2 kD, V_e), which were neutralized with 1 M NH_4OH and lyophilized. An aqueous solution of the test material in a volume of 10 µl was injected intracisternally into a group of intact recipients, consisting of 10-12 rats. PA was recorded by the method in [6]. Material was considered to be active if, after injection, the fraction of animals with flexion of one hind limb, the right for example, was significantly larger than the fraction of animals with flexion of the left limb. In that case the PAF was called right-sided. PAF inducing flexion of the left limb was called left-sided PAF. The significance of differences was determined statistically by Fisher's exact method [5]. Activity of PAF was assessed as the number of minimal active doses per milligram of tissue.

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