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COMPARATIVE ANALYSIS OF THE ROLE OF β-ENDORPHIN SYSTEMS IN MECHANISMS OF DIFFERENT TYPES OF ANALGESIA

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Experiments using the opiate antagonist naloxone have shown that the development of analgesia under the influence of stress stimuli, acupuncture, and electrical stimulation of brain structures may arise through the intervention of opioid and other neurochemical mechanisms [1-10]. However, there is evidence that opioid systems are not always involved in the mechanisms of analgesia [6, 10], evidently because of selective activation of neurochemical components during exposure to certain types of action.

Accordingly, in the investigation described below, which was conducted on animals immunized with a conjugate of β-endorphin (EN) and bovine serum albumin (BSA), in order to inhibit activity of the EN-system, a comparative analysis was made of the role of this system in mechanisms of analgesia in different situations.

EXPERIMENTAL METHOD

Experiments were carried out on 36 albino rats. To depress activity of the EN system 18 rats were immunized by two injections (at an interval of 7 days) of 0.1 ml of conjugate, mixed in the ratio of 1:1 with Freund's adjuvant, into the upper third of the hind limb. The conjugate was prepared in a reaction mixture of EN—BSA—bis-diazotized benzidine (10:1:10), and the efficiency of the conjugation reaction was 60% (i.e., EN:BSA = 6:1). The final concentration of EN was 75 μg in 0.1 ml. Rats of the control group (n = 18) were given an injection of 0.1 ml of an unconjugated mixture (UCM) of EN—BSA—Freund's adjuvant in the same proportions.

Experiments were carried out 8 days after the second immunization. Nociceptive sensitivity was assessed by studying latent periods (LP) of paw licking responses (PLR) to a hot plate at 55°C and the tail withdrawal response (TWR) to the same thermal stimulation. Anesthesia was induced by intraperitoneal injection of morphine (5 mg/kg), by swimming in cold water at 4°C for 3 min, or by unavoidable electric shock stress (ESS). The animals were exposed to ESS in a chamber with an electrically conducting floor, through which a continuous pulsed current was passed (2.5 mA, 8 pulses/min, 2 sec, 10 min).

The rats were decapitated 3-4 days after the experiment. To determine the EN concentration the pituitary and hypothalamus were isolated from the brain and kept at −30°C. EN was extracted by the method in [8]. The EN concentration was determined by radioimmunoassay, using reagents from Immuno Nuclear Inc. (USA).

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TABLE 1. Time Course of Changes in LP of PLR and TWR in Rats Immunized with EN Conjugate or with UCM, after Injection of Morphine, ESS, or Cold Stress (CS; M ± m)

Response tested and experimental conditions	Initial LP, sec	Time (in min) after exposure					
		0	5	10	20	30	40
PLR							
Conjugate + morphine	13,6±1,6			30,0±5,9[a]	35,0±4,9[a]	33,1±4,5[a]	34,8±5,2[a]
UCM + morphine	14,7±1,8			29,9±4,6[a]	35,8±4,6[a]	29,6±3,6[a]	28,3±3,8[a]
TWR							
Conjugate + morphine	3,8±0,3			5,2±0,8	4,5±0,5	4,8±0,6	4,9±0,6
UCM + morphine	3,9±0,6			7,0±0,4[a,b]	6,7±0,4[a,b]	5,3±0,7	5,5±0,9
PLR							
Conjugate + ESS	12,5±0,6	30,4±4,3[a]	28,7±5,2[a]	26,7±3,8[a]	20,2±6,2	19,3±4,9	20,8±6,3
UCM + ESS	10,7±0,8	36,8±0,3[a]	31,5±3,9[a]	26,5±3,5[a]	17,4±2,9[a]	16,2±2,7	20,8±4,4[a]
TWR							
Conjugate + ESS	4,3±0,4	5,0±0,7	4,2±0,35	4,3±0,44	3,5±0,33	3,5±0,28	2,9±0,31
UCM + ESS	4,3±0,3	6,4±0,4	6,6±0,3[a,b]	5,8±0,6[a,b]	6,0±0,5[a,b]	5,0±0,5	4,3±0,5
PLR							
Conjugate + CS	20,3±0,9	40,0±0,0[a]	40,0±0,0[a]	40,0±0,0[a]	40,0±0,0[a]	36,9±3,1[a]	40,0±0,0[a]
UCM + CS	19,4±0,2	40,0±0,0[a]	40,0±0,0[a]	40,0±0,0[a]	38,4±1,6[a]	40,0±0,0[a]	36,9±3,1[a]
TWR							
Conjugate + CS	3,7±0,3	6,7±0,3[a]	5,7±0,6[a]	6,7±0,3[a]	6,7±0,3[a]	5,9±0,4[a,b]	6,8±0,2[a]
UCM + CS	4,2±0,3	7,0±0,0[a]	6,4±0,5[a]	6,1±0,6[a]	6,5±0,4[a]	6,6±0,4[a]	6,3±0,4[a]

Legend. a) $p < 0.05$ compared with LP before and after exposure, b) $p < 0.05$ compared with LP in experimental (conjugate) and control (UCM) groups.

EXPERIMENTAL RESULTS

Biochemical determination of EN in tissues of the pituitary and hypothalamus showed a decrease in its concentration in rats immunized with the conjugate compared with the group of rats receiving UCM. For instance, the EN concentration in the pituitary of the first group of rats was $(3.42 \pm 0.34) \times 10^{-9}$ mole/mg, and $(4.89 \pm 0.46) \times 10^{-9}$ mole/mg in the second group ($p < 0.05$). The EN concentration in the hypothalamus of rats of the experimental group was lower ($p < 0.05$) than in the control: $(88.9 \pm 13) \times 10^{-15}$ and $(206.6 \pm 26) \times 10^{-15}$ mole/mg, respectively. These results are evidence that immunization of rats with the conjugate induces marked inhibition, though only partial, compared with the control, of activity of the brain EN systems.

Values of LP of PLR and TWR for rats receiving the conjugate or UCM are given in Table 1. The initial value of LP in the different series of experiments showed no significant differences in rats of the experimental and control groups. It can accordingly be concluded that the EN-systems of the brain do not affect sensitivity to pain in the resting state. This conclusion is in agreement with data showing that the antagonist naloxone does not change the background level of sensitivity to pain [7]. Meanwhile, since as a result of immunization of the animals the EN-systems were not completely blocked, it can be tentatively suggested that the background level of sensitivity to pain is maintained by the low activity of these systems.

The results of experiments to study the time course of LP in response to intraperitoneal injection of morphine are given in Table 1 also. In animals of the experimental ($n = 6$) and control ($n = 6$) groups morphine induced a significant increase in LP of PLR, after 10 min, compared with the initial period, and this persisted for 40 min. On statistical comparison of LP in the experimental and control groups no significant differences were found.

Comparison of LP of TWR gave the following results. In the experimental group of rats morphine caused a small but not significant increase in LP compared with the initial level at all times of the experiment (Table 1). However, in the control group, a significant lengthening of LP of TWR compared with the initial period was observed after 10 and 20 min ($p < 0.001$ and $p < 0.01$, respectively) and with the control ($p < 0.05$ and $p < 0.001$). Hence it can be concluded that the morphine-activated mechanisms of analgesia are maintained not only by interaction between the analgesic and opiate receptors. EN-systems, which are evidently activated by morphine, also play an important role in these processes.

In the next series of experiments the time course of the change in sensitivity to pain during ESS was studied. In rats of the experimental group ($n = 6$) ESS induced an increase in LP of PLR compared with the initial period, but only for 10 min (Table 1). In the control group ($n = 6$), however, lengthening of LP was observed for 40 min of the recovery period.

However, comparison of the results showed no significant differences between LP of PLR in the experimental and control groups. Analysis of the duration of LP of TWR showed a very small (not significant) increase in LP in the rats after immunization with conjugate and in response to ESS, only after 5 min. Later, LP were initially shorter, and starting from the 20th minute they became shorter, although not significantly, than before ESS. By contrast, in rats of the control group, LP after stress were significantly longer than initially, for 20 min.

Comparative analysis showed that in rats of the experimental group LP of TWR after ESS was significantly shorter than in the control, starting from the 5th minute. Hence it follows that the EN-systems play an important role in the mechanisms of analgesia in pain stress.

In the next series of experiments to study LP of PLR and TWR of rats swimming in cold water, an increase in the nociceptive responses was observed in both groups (Table 1). Under these circumstances no differences were found between LP of the experimental (n = 6) and control (n = 6) rats. It can accordingly be concluded that the EN-systems are not involved in the antinociceptive process in cold stress.

The experiments thus indicate that immunization of rats twice with conjugate of EN and BSA is followed by a fall in the EN concentration in the pituitary and hypothalamus. It can also be concluded from these results that regulation of sensitivity to pain arising as the result of exposure to different factors is maintained by mechanisms which differ in their neurochemical composition.

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