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EFFECT OF REPEATED ACUPUNCTURE ON NOCICEPTION AND ON β-ENDORPHIN LEVELS IN THE RAT HYPOTHALAMUS AND MIDBRAIN

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Many investigations have shown that acupuncture (AP) is accompanied by the development of marked analgesia in man and animals [1-6, 9]. The development of this phenomenon has been explained by activation of the opioid systems of the brain, as shown by indirect pharmacological and direct biochemical evidence. It has been shown, for instance, that the opiate antagonist naloxone inhibits the development of the analgesic effect of AP [1, 3, 4, 6, 9]. Weakening of nociception after AP is accompanied by changes in opioid substances in the brain and cerebrospinal fluid [1, 6]. All these data were obtained in experiments with single AP. The problem of the effect of repeated AP on regulation of nociception and on the opioid peptide level in various brain formations has not yet been studied.

It was accordingly decided to study the background level of nociception, the development of analgesia, and the β-endorphin (βE) concentration in the midbrain and hypothalamus of rats subjected to repeated AP.

EXPERIMENTAL METHOD

Experiments were carried out on 59 albino rats weighing 200-250 g. Animals of the experimental group (23 rats) underwent a daily session of AP lasting 15 min for 15 days. AP was given by insertion of needles into the acupuncture zone in the region of the Ho-Ku point on both forelimbs. Rats of the control group were not subjected to AP sessions. Nociception was evaluated before the experiment began and on the 1st, 3rd, 5th and 15th days before and after AP. The parameters tested in the control rats were measured at the same time intervals. Nociception was determined as the latent periods (LP) of the paw licking response (PLR) to placing the rats on a hot plate at 55°C and the tail withdrawal response (TWR) to focusing a beam of light from a 150-W lamp on it.

After the experiments the animals of both groups were decapitated and the hypothalamus and midbrain were removed by the method in [7]. βE was extracted from the tissue with 0.2 N hydrochloric acid (w/v = 1/10), during boiling on a water bath for 10 min, followed by homogenization of the samples. The homogenates were centrifuged at 10,000g for 15 min. The supernatant was frozen and lyophilized, and the dry extract was kept at −30°C. The βE concentra-

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TABLE 1. Values of Background LP of PLR and TWR (in sec) in Rats of Control Group and after AP Sessions

Experimental conditions	Number of AP			
	0	3	5	15
		PLR		
Control	14,3±0,8	13,4±1,4	—	11,1±1,0
Experiment	14,1±0,8	17,9±2,9	18,3±2,9	30,9±2,5[b,f]
		TWR		
Control	3,2±0,1	2,4±0,1[a]	—	2,6±0,3[c,e,d]
Experiment	3,1±0,1	4,0±0,4[c,d]	4,0±0,4	3,9±0,5

Legend. [a] $p<0.005$, [b] $p<0.05$, [c] $p<0.01$ compared with initial level (Dunnett's test); [d] $p<0.05$, [e] $p<0.002$, [f] $p<0.001$ compared with control (Student's t test).

TABLE 2. Changes in Nociceptive PLR (in % of initial) in Rats during AP

Parameter	Number of AP			
	1	3	5	15
PLR	59,2±13,5 $p<0,001$	—18,0±5,8[c] $p<0,01$	11,4±17,5[c]	—0,14±6,6[b]

Legend. [a] $p<0.05$ (1α), [b] $p<0.01$, [c] $p<0.005$ compared with 1st session (Dunnett's test). $p < 0.01$ and $p < 0.001$ For Student's paired t test.

tion in the tissue extracts was determined by radioimmunossay, using commercial kits of reagents for βE assay (Immuno Nuclear Corp., USA).

The results were subjected to dispersion analysis. The significance of differences was estimated by the tests of Dunnett and Duncan, and in some cases, by Student's t test.

EXPERIMENTAL RESULTS

The results showed that initial values of LP of PLR and TWR of animals of the experimental and control groups were virtually indistinguishable (Table 1). Comparison of the background values of LP of PLR in animals of the experimental group by dispersion analysis showed a difference between parameters measured on different days of the experiment ($F_{3,39} = 13.8$; $p<0.002$). After 3 and 5 sessions of AP, the rats showed an increase (not significant) in LP of PLR compared with the initial value, and after the 15th session, the increase was significant (Dunnett's t criterion 5.79; $p<0.005$), the LP of PLR being approximately doubled. It was also shown that the duration of LP at this time of the experiment was greater ($p<0.001$) than at all other times. By contrast with the experimental rats, LP of PLR of the animals of the control group, measured at the same times, was shorter than initially, but the decrease was not significant ($F_{2,36} = 2.06$; $p>0.05$). Subsequent statistical analysis showed that on the 15th day LP of PLR of the experimental rats was longer than in the controls ($p<0.001$). Qualitatively similar results were obtained by measurement of LP of TWR. In rats of the experimental group, for instance, LP was longer than in the control on the 3rd and 15th days ($p<0.002$ and $p<0.05$ respectively). Such an increase in the duration of LP compared with initial and control values indicates an increase in tone of the antinociceptive mechanisms controlling the state of background nociception in rats subjected to repeated AP. Similar results were obtained in experiments [5, 8] to study mechanisms of chronic unavoidable pain stress.

The results given in Table 2 characterize the duration of LP of LPR before and after different numbers of AP sessions and they enable the state of the antinociceptive systems to be assessed in response to the action of this type of analgesia. The results show that an increase ($p<0.001$) of LP of PLR by 60% compared with a background level was found only after the first AP session. At later stages of the experiment the duration of LP was not increased, but was redueed ($p<0.01$) by 20% compared with the background value on the 3rd day, and was indistinguishable from it on the 5th and 15th days. At the same time it was shown that the increase in LP, observed at the first session was greater ($F_{3,53} = 11.06$; $p<0.002$) than after all other time intervals. It can be concluded from these results that repeated AP leads to

TABLE 3. βE Concentration ($\times 10^{-15}$ mole/mg) in Midbrain and Hypothalamus of Control Rats and after AP

Test object	Control	Number of AP		
		3	5	15
Midbrain	8,0±0,2	6,8±0,3[b,d]	8,3±0,4	6,8±0,4[d]
Hypothalamus	117,8±6,1	139,4±4,4[a,e]	97,5±7,9	146,0±5,7[c,e]

Legend. [a] $p<0.05$, [b] $p<0.025$, [c] $p<0.01$ compared with control (Dunnett's t test); [d] $p<0.05$, [e] $p<0.01$ compared with 5th session (Duncan's test).

weakening of the ability of the antinociceptive mechanisms to become activated in response to AP. A similar phenomenon also was observed in experiments on rats subjected to chronic stress.

One cause leading to inhibition of the mechanisms of evoked activation of antinociception may be a change in βE metabolism in the hypothalamus and midbrain, which are involved in these processes.

Data showing βE levels in tissues of the hypothalamus and midbrain in rats of the experimental and control groups are given in Table 3. The concentration of the peptide in tissues of animals of the control groups, corresponding to particular times of application of AP, were virtually identical. The βE concentration in the midbrain was reduced below the control level after the 3rd session, increased after the 5th, and reduced again after the 15th session (Table 3). Dispersion analysis of the βE level demonstrated significant differences between all groups of rats ($F_{3,32} = 50.5$; $p<0.002$). Dunnett's postdispersion comparison revealed a significant fall in the βE concentration compared with the control after the 3rd and 15th sessions by 15% ($t = 2.5$, $p<0.025$) $t = 2.61$, $p<0.025$ respectively), whereas after the 5th session of AP the βE concentration did not differ significantly from the control. Comparison of this parameter in the groups of experimental rats at different stages of the investigation revealed significant differences between them ($F_{2,17} = 5.01$; $p<0.05$). The concentration of βE after five sessions of AP was significantly higher than after the 3rd and 15th sessions (Duncan's $t = 4.16$, $p<0.05$ and $t = 4.02$, $p<0.05$ respectively).

The time course of the βE level in the hypothalamus was opposite in character to that in the midbrain. For instance, an increase was observed in the concentration of the peptide after the 3rd and 15th sessions (by 18 and 39%) and a decrease to the control value after the 5th session. The existence of significant differences in the groups of control and experimental rats was established by single-factor analysis ($F_{3,30} = 8.91$, $p<0.002$). The βE concentration was found to be significantly higher than in the control after the 3rd and 15th sessions (Dunnett's $t = 2.2$, $p<0.05$ and $t = 2.87$, $p<0.01$ respectively). Comparison of the parameter in the experimental groups of rats also revealed significant ($F_{2,17} = 17.79$, $p<0.002$) differences between them. Duncan's postdispersion analysis revealed a significantly higher βE concentration on the 3rd and 15th days than on the 5th day ($t = 7.95$ and 6.86; $p<0.001$).

The results are thus evidence that the time course of βE in the hypothalamus and midbrain is opposite in direction, with maximal changes after the 3rd and 15th sessions of AP, whereas after the 5th session βE was identical with the control values. In all probability this change of relations between activity of the βE systems of the hypothalamus and midbrain is the cause of development of adaptation of the antinociceptive mechanisms regulating the state of nociception both in the background and in response to AP.

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