ACTION OF AN ENKEPHALINASE BLOCKER ON EFFECTS OF ACUPUNCTURE IN RABBITS SENSITIVE AND RESISTANT TO IT

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Acupuncture analgesia, induced by stimulation of both corporal and auricular points with a frequency of 1-30 Hz, is known to be blocked by naloxone [2, 3, 6], and under these circumstances the concentrations of endorphins and enkephalins in the CSF and in brain homogenate are increased, but this phenomenon is absent in acupuncture-resistant animals [9]. It is recognized that these opioid peptides also are responsible for the analgesic effect of acupuncture. However, the mechanism of reuptake of the peptides has not been discovered and their biological inactivation takes place with the aid of a system of peptide-hydrolase activation [1]. Consequently, it might be supposed that restoration of normal sensitivity to pain after acupuncture analgesia takes place through the last mechanism of inactivation of an excess of opioid peptides.

In this investigation, in order to elucidate this mechanism we studied changes in sensitivity to pain during acupuncture stimulation in acupuncture-sensitive and resistant rabbits after administration of D-phenylalanine (d-Phe), a blocker of brain enkephalinase [4].

EXPERIMENTAL METHOD

Experiments were carried out on 30 conscious male chinchilla rabbits, lightly restrained by their paws, weighing 3-3.5 kg, and scalped under local anesthesia beforehand. As a test of pain sensitivity in the rabbits we used changes in the evoked potential (EP) in response to electrical stimulation of the dental pulp [2, 3]. EP were recorded in the somatosensory cortex, and their analysis, especially changes in values of the amplitude of the negative–positive (N_1P_2) components of EP with a latent period (LP) of 20-40 msec in response to electrodental stimulation (EDS), and also auriculo-acupuncture electrical stimulation (AAE) with a frequency of 15 Hz for 25 min, were carried out by methods described previously [3]. D-Phe, in a dose of 250 mg/kg [7], in aqueous solution, was injected intraperitoneally.

EXPERIMENTAL RESULTS

In response to EDS, an EP with LP of the primary positive wave of between 7 and 12 msec and an amplitude of 50-70 μV was recorded in the sensomotor cortex of the rabbits in response to EDS, and it was followed by a negative–positive wave (N_1P_2) with LP of the negative peak of 20-30 msec, and of the positive peak of 35-40 msec; their amplitude increased with an increase in the strength of the current used for EDS (Fig. 2) and it was relatively constant (differences not significant) if the strength of the EDS current was constant, as was the case during background recording for 15-20 min.

Application of AAE caused a decrease in amplitude of the N_1P_2 component of EP in 19 of the 30 rabbits in response to EDS with the same strength of current on average to 58 ± 7% of the initial values, and this was maintained with very small fluctuations for 40-50 min after discontinuation of AAE, followed by an increase in the values up to 89 ± 9% by the 70th minute and 108 ± 11% toward the 80th minute after discontinuation of AAE.

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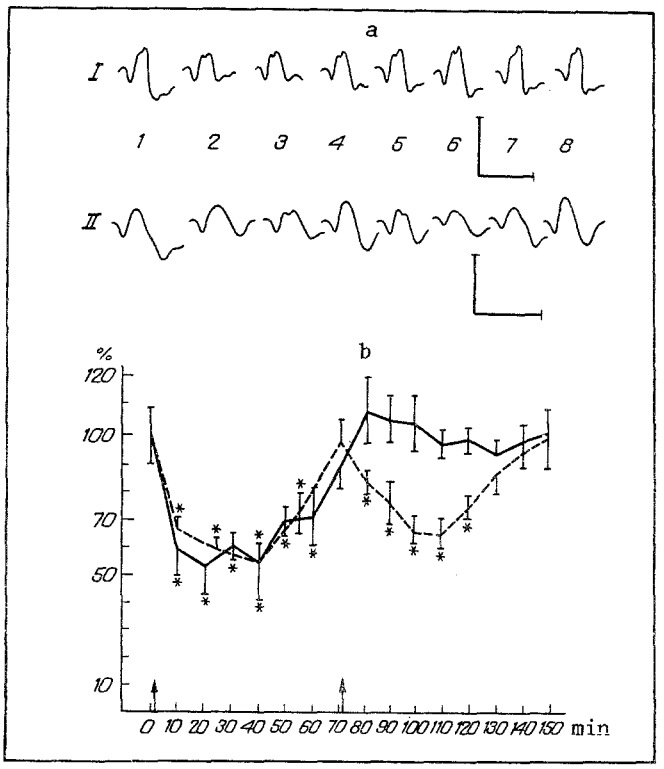

Fig. 1. Time course of somatosensory cortical EP of rabbit in response to EDS: a) change in EP of rabbit somatosensory cortex in response to EDS: I) before (1) and 10 (2), 20 (3), 40 (4), 60 (5), 80 (6), 120 (7), and 140 (8) min after AAE; II) before (1) and 10 (2), 20 (3), and 40 (4) min after AAE and 20 (5), 40 (6), 60 (7), and 80 (8) min after subsequent injection of D-Phe. Calibration: 50 μV, 50 msec. b) Change in amplitude of N_1P_2 component of somatosensory cortical EP in response to EDS in acupuncture-sensitive rabbits as a percentage of initial level (100%) after AAE (indicated by 1st arrow) in control group of rabbits (continuous line) and after injection of D-Phe (2nd arrow) in experimental group of animals (broken line). Abscissa, time (in min); ordinate, amplitude of component N_1P_2 of EP (in % of initial values, taken as 100%). Asterisk indicates significance of differences compared with initial values.

During the next 70 min, no significant differences were observed (n = 10) between the amplitude of this component of EP in response to EDS compared with the initial values before AAE (Fig. 1). During the next experiments (n = 5) without the use of AAE, injection of D-Phe into acupuncture-sensitive rabbits caused no significant changes in the amplitude of the N_1P_2 component of EP in response to BDS during 75 min of observation (Fig. 2).

In later experiments application of AAE to acupuncture-sensitive rabbits (n = 10) caused a decrease in the amplitudes of the N_1P_2 component of the EP in response to EDS, similar to that described above, for 50-60 min, followed by recovery to 96 ± 8% of the original values by the 70th minute after discontinuation of AAE. Injection of D-Phe at this time caused a decrease in amplitude of the N_1P_2 component of EP to 83 ± 4% after 10 min, to 75 ± 8% after 20 min, and to 66 ± 5% after 30-40 min, which was followed by a gradual increase to values not differing significantly from those observed initially, 60-70 min after injection and 80-130 min after discontinuation of AAE (Fig. 1).

In experiments on 11 rabbits application of AAE caused no significant changes in the amplitude of the N_1P_2 component of EP in response to EDS during observation for 75 min after discontinuation of AAE (Fig. 2). Injection of D-

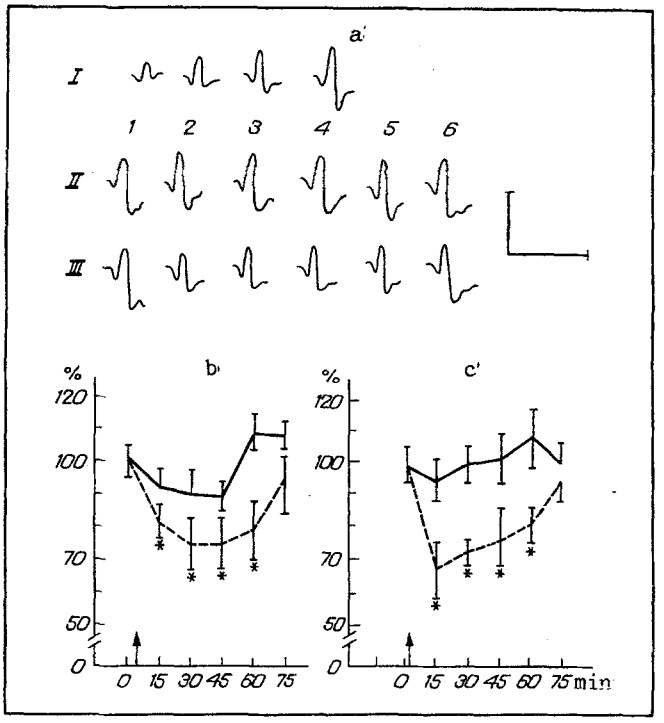

Fig. 2. Dynamics of EP of rabbit somatosensory cortex in response to EDS: a) changes in EP of rabbit somatosensory cortex in response to EDS: I) EDS with a current of 1 mA (1), 3 mA (2), 5 mA (3), and 7 mA (4); before (1), and 15 (2), 30 (3), 45 (4), 60 (5), and 75 (6) min after injection of D-Phe, in acupuncture-sensitive (II) and acupuncture-resistant (III) rabbits. Calibration: 50 μV, 50 msec. b) Changes in amplitude of N_1P_2 component of EP (in % of initial level) in acupuncture-sensitive (continuous line) and acupuncture-resistant (broken line) rabbits after injection of D-Phe (arrow). c) Change in amplitude of N_1P_2 component of EP (in % of initial level) in acupuncture-resistant rabbits after AAE (arrow) without injection (continuous line) and with injection of D-Phe 5 min before AAE (broken line). Legend as to Fig. 1.

Phe into these acupuncture-resistant animals (n = 5) without application of AAE caused a significant decrease in the amplitude of the N_1P_2 component of EP in response to the same EDS to 82 × 5% after 15 min, to 75 ± 8% after 30 and 45 min, and to 79 ± 9% after 60 min, followed by recovery to 94 ± 9% of the initial value 75 min after injection (Fig. 2).

In the next experiments (n = 10) injection of D-Phe 5 min before application of AAE caused a significant reduction of the amplitude of the N_1P_2 component of EP in acupuncture-resistant rabbits 15 min after discontinuation of AAE in response to EDS to 68 ± 9% of the initial value, after 30 min to 73 ± 4%, after 45 min to 76 ± 8%, and after 60 min to 81 ± 6%, with recovery to 96 ± 7%, followed by no significant difference from the initial values toward 70-75 min after discontinuation of AAE or to 100-105 min after injection of D-Phe (Fig. 2).

The results of these experiments thus snowed that in 64% of the animals AAE caused a significant decrease in the amplitude of the N_1P_2 component of the somatosensory cortical EP in response to EDS, followed by gradual recovery to the initial values 50-70 min after discontinuation of AAE, a result which can be interpreted as the manifestation of an analgesic effect [2, 3], and the animals concerned can be regarded as acupuncture-sensitive. In 36% of animals application of AAE did not cause this analgesic effect, and these animals were classed as acupuncture-resistant.

Isolated injection of the enkephalinase blocker D-Phe, thereby increasing the enkephalin concentration in the brain [4], caused no changes in nociception in acupuncture-sensitive animals However, its injection at the stage of recovery of nociception after acupuncture analgesia did induce an analgesic effect, i.e., prolongation of the analgesic effect of AAE by 40-50 min, in agreement with the results of observations made in man [5]. This is evidence that during recovery of nociceptive sensation after acupuncture analgesia the enkephalinase mechanism of inactivation of endogenous opioids is activated, However, the absence of an effect of D-Phe in acupuncture-sensitive animals, unaccompanied by application of AAE is evidence that under ordinary conditions the enkephalinase mechanism is not activated, and its activation is probably linked with a definite level of increase of the opioid concentration in structures of the CNS.

Meanwhile isolated injection of D-Phe into acupuncture-resistant animals caused weakening of nociception with gradual restoration of the initial level 30-40 min after injection, whereas combined administration of D-Phe and AAE intensified and prolonged its analgesic effect. Thus enkephalinase blockade itself induced an analgesic effect in acupuncture-resistant rabbits, which at the same time became acupuncture-sensitive. A similar effect of D-Phe injection also has been observed in acupuncture-resistant rats [7].

It can be postulated on the basis of these observations that the primary cause of acupuncture-resistance is not a low concentration of endorphins and enkephalins in the CNS [9, 8], but a constant high activity of the enkephalinase mechanisms, causing inactivation of endogenous opioids [1], which prevents realization of the analgesic effect of acupuncture, which is mediated through activation of the endogenous opioid system.

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