EFFECT OF LEUCINE-ENKEPHALIN ON CEREBRAL CORTICAL UNIT ACTIVITY

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Experiments on unanesthetized rats immobilized with flaxedil showed that leucine-enkephalin (150 μg), if injected into the lateral cerebral ventricle, caused heterogeneous changes in the activity of 21 sensomotor cortical units tested. Spike discharges of five neurons were reduced and those of 11 neurons increased. Naloxone (2 mg/kg, intravenously) prevented both the inhibitor and the activating effect of leucine-enkephalin in the case of repeated injections of this pentapeptide. Leucine-enkephalin did not change the activity of five units.

KEY WORDS: leucine-enkephalin; naloxone; sensomotor cortex; changes in unit activity.

It is generally considered that the effects of narcotic analgesics are determined by their specific action on opiate receptors [7, 12]. The endogenous ligands methionine-enkephalin and leucine-enkephalin also possess high stereospecificity for opiate receptors [9]. Meanwhile the localization of the analgesic action of preparations of the morphine and enkephalin group has not yet been finally established, for opiate receptors are widely distributed in various parts of the CNS, including the cerebral cortex [3]. The concentration of enkephalins in the CNS likewise varies. It has been shown that the concentration of leucine-enkephalin is relatively higher than that of methionine-enkephalin in the cerebral cortex [10]. The object of the present investigation was accordingly to study the effect of leucine-enkephalin on cortical unit activity.

EXPERIMENTAL METHOD

Experiments were carried out on rats weighing 250-300 g. The preliminary manipulations (trephining the skull, tracheotomy, catheterization of the veins and artery) were carried out under ether anesthesia. The animals were then fixed in a special frame, immobilized with flaxedil (10 mg/kg, intravenously), and artificially ventilated. Activity of groups of sensomotor cortical neurons was recorded extracellularly with glass microelectrodes filled with 2.5 M KCl solution (tip 1.5-2 μ in diameter), 1.5 h after the end of inhalation of ether. The signal was led to a type MZ-4 amplifier with high input resistance. Suppression of cophased interference and detection and amplification of the signal were carried out by means of a type U2-8 measuring amplifier. The data on unit activity were processed on a PP-15A scaler, connected through a threshold device with the output of the measuring amplifier. Unit activity was analyzed graphically on a multichannel N-338 automatic plotter, by means of a program of transformation of numerical information into pulses of proportional amplitude. Leucine-enkephalin was injected in a dose of 150 μg in 10 μl of Ringer's solution from a Hamilton microinjector into the lateral ventricle of the rats (coordinate: V = 3.5 mm, A = 2 mm, L = 2 mm from the bregma) for 30-45 sec. Leucine-enkephalin, when injected into the cerebral ventricle in this dose, is known [4] to produce analgesia in rats during temperature stimulation of the tail. Naloxone (2 mg/kg, intravenously) was used as antagonist of leucine-enkephalin. Response of 21 neurons in 10 rats was studied. Control experiments showed that repeated injection of Ringer's solution in a dose of 10-15 μl into the lateral ventricle did not cause changes in unit activity.

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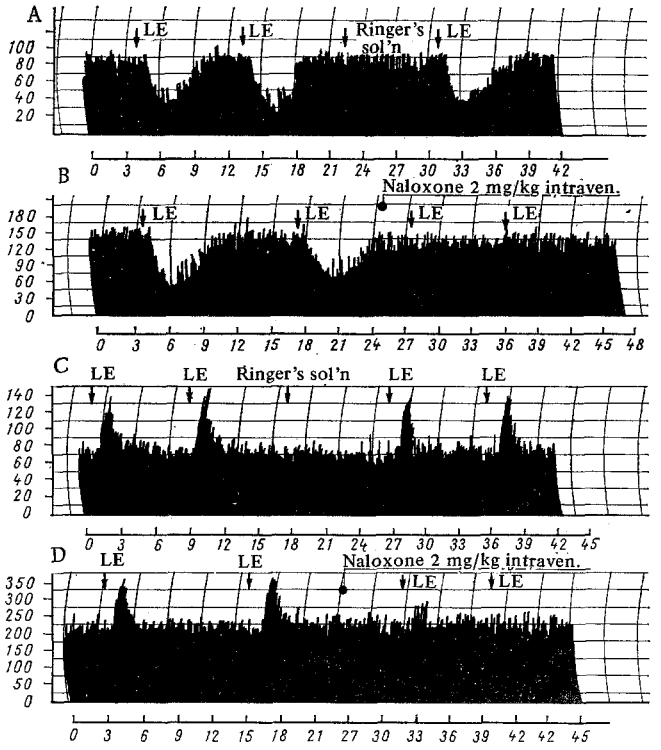

Fig. 1. Changes in sensomotor cortical unit activity after injection of leucine-enkephalin (150 μg) into the lateral ventricle in rats. A) Inhibitor action of leucine-enkephalin on unit activity; B) antagonism between naloxone and leucine-enkephalin; C) activating action of leucine-enkephalin on unit activity; D) antagonism between naloxone and leucine-enkephalin. Vertical lines indicate discharge frequency of neurons (spikes/sec), horizontal lines, time (in min). Arrows indicate times of injection of drugs. LE) Leucine-enkephalin.

EXPERIMENTAL RESULTS AND DISCUSSION

The effect of leucine-enkephalin on sensomotor cortical neurons varied. For instance, leucine-enkephalin reduced the discharge frequency of five neurons by 42-56% of the initial level ($P<0.01$). The maximal effect developed 3-4 min after administration of the substance, and after 5-8 min unit activity had returned to its initial level. During repeated injections of leucine-enkephalin, the activity of these cells also was inhibited (Fig. 1A). Techyphylaxis did not develop after three to five injections at intervals of 10-15 min. Meanwhile naloxone prevented changes in unit activity during repeated injections of leucine-enkephalin (Fig. 1B). Activity of 11 units was increased by 52-60% under the influence of leucine-enkephalin ($P<0.01$). The action reached a maximum 3-4 min after injection of the leucine-enkephalin, and the original spike discharge frequency was restored after 5-8 min (Fig. 1C). In this case the direction of the action of leucine-enkephalin when injected repeatedly remained the same as before, and no evidence of tachyphylaxis was present. Naloxone also prevented the activating action of repeated injections of leucine-enkephalin (Fig. 1D). The discharge frequency of five neurons was unchanged after injection of leucine-enkephalin into the lateral ventricle.

The investigation thus showed that leucine-enkephalin has a variable action on sensomotor cortical neurons in rats after intraventricular injection. The action of leucine-enkephalin on neurons sensitive to it is evidently specific, for both the inhibitory and the activating effects of this morphine-like polypeptide were prevented by injection of naloxone, an antagonist of narcotic analgesics. Preparations belonging to the group of naroctic analgesics act in different ways on neurons in different parts of the CNS. Morphine, for example, differs in its action on spinal neurons depending on whether it is administered systemically [2] or by microiontophoresis [5]. A study of the effect of enkephalins on cortical and brain-

stem neurons likewise has revealed both an inhibitory and an activating action of these substances when applied by microiontophoresis [6]. Naloxone blocked the above-mentioned effects of endogenous morphine-like compounds. Davies and Dray [6], in particular, attribute differences in the sensitivity of neurons to the possibility that different opiate receptors may exist in the CNS. Heterogeneous changes in unit activity are also observed during electrical stimulation of the raphe nuclei [1], which is known [8] to cause analgesia in animals, possibly on account of an increase in the concentration of endogenous morphine-like peptides in the CNS.

It can thus be tentatively suggested that analgesia connected with an increase in the level of endogenous morphine-like peptides in the CNS following stimulation of the raphe nuclei and also following injection of enkephalins or preparations of the morphine group from an outside source, is accompanied by disturbances of functional relations between neurons of both subcortical and cortical formations. This hypothesis is supported by data showing that sensomotor cortical cells participate in responses to nociceptive stimulation [11].

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THE ROLE OF CALCIUM IN THE MECHANISM OF CHANGES IN VASCULAR REACTIVITY DUE TO RESERPINE

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Under the influence of reserpine there is a marked decrease in the calcium concentration in segments of the distal aorta and femoral arteries of rabbits, incubated in salt solution. These changes develop parallel with changes in the character of responses of the vascular segments to direct electrical stimulation (weakening of the effects of contraction, followed by relaxation). A decrease in the calcium concentration in the vascular wall is suggested as one cause of the change in vascular reactivity.

KEY WORDS: calcium; reserpine; reactivity of blood vessels.

It has been concluded from experimental data obtained during a study of the effect of reserpine on neurogenic vasomotor responses that the fall in blood pressure is due to modified vascular reactivity to nervous influences [1, 4, 5]. Changes in the reactive properties of the blood vessel wall under the influence of reserpine, manifested as the replacement of contraction by relaxation, have been confirmed by recording contractile responses of isolated vascular segments to direct, measured electrical stimulation [3]. Reserpine is a sympatholytic drug which causes exhaustion of the tissue noradrenalin reserves [13]. However, this

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