3. V. I. Pogadaev, E. N. Timin, and B. I. Khodorov, Biofizika, 21, 848 (1976).
4. R. Andersson and E. Mohme-Lundholm, Acta Physiol. Scand., 79, 244 (1970).
5. C. Mayer, C. Van Breemen, and R. Casteels, Pflug. Arch. Ges. Physiol., 337, 333 (1972).
6. G. Pöch and W. Umfahrer, Arch. Exp. Path. Pharmak., 293, 257 (1976).

EFFECT OF LEUCINE-ENKEPHALIN ON INTERNEURONAL TRANSMISSION OF EXCITATION

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Experiments on unanesthetized cats immobilized with flaxedil showed that injection of leucine-enkephalin (1 mg) into the lateral ventricle is followed by inhibition of evoked responses in the ventrolateral columns of the spinal cord and of segmental interneuronal transmission in the spinal cord and by a decrease in the amplitude of potentials in cortical somatosensory area I evoked by sciatic nerve stimulation. Naloxone (1 mg/kg, intravenously) prevented these effects of leucine-enkephalin. Preliminary injection of methysergide (2.5 mg/kg, intraperitoneally) led to weakening of the effect of leucine-enkephalin on spinal interneuronal transmission. Leucine-enkephalin did not change the amplitude of polysynaptic potentials of the glossomandibular reflex, the arc of which is closed in the brain stem.

KEY WORDS: leucine-enkephalin; naloxone; interneuronal transmission of excitation; methysergide.

Narcotic analgesics are known to influence interneuronal transmission of excitation at different levels of the CNS [1-3]. Analgesia is produced by these substances through their specific interaction with opiate receptors, present in various regions of the CNS [13]. Serotoninergic mechanisms also take part in the development of the analgesic effect of drugs belonging to the morphine group [15]. The pentapeptides leucine-enkephalin and methionine-enkephalin are endogenous neuromediators with morphine-like activity [7, 11]. Electrical stimulation of the raphe nuclei induces analgesia [9], which is evidently connected with an increase in the liberation of polypeptides with morphine-like activity [6]. There is thus a definite parallel between the effects of narcotic analgesics and of morphine-like polypeptides.

The object of this investigation was to study the effect of one morphine-like polypeptide, namely leucine-enkephalin, on interneuronal transmission of excitation in the CNS, on the character of its interaction with naloxone, a specific antagonist of the narcotic analgesics, and with methysergide, which blocks serotoninergic receptors.

EXPERIMENTAL METHOD

Experiments were carried out on unanesthetized cats of both sexes, weighing 2.8-3.5 kg, immobilized with flaxedil (2 mg/kg, intravenously). The preliminary manipulations – dissection of the sciatic nerve, spinal cord, anterior roots of the cord at the level of segments L7-S1, trephining of the skull, and catheterization of veins and arteries – were carried out under ether anesthesia. Artificial ventillation was provided by the DP-5 apparatus at the rate of 205 ml air/kg body weight·min [8]. Evoked potentials, in response to stimulation of the central end of the divided sciatic nerve with single supramaximal (5-12 V) pulses of current 0.1 msec in duration, were recorded in cortical somatosensory area I on the contralateral side, and also in the ventrolateral columns of the spinal cord at the level of segments L2-L3 on both ipsi- and contralateral sides. Monosynaptic and polysynaptic responses in the anterior roots of the spinal cord during analogous sciatic nerve stimulation and polysynaptic potentials in the central structures of the glossomandibular reflex, arising in the

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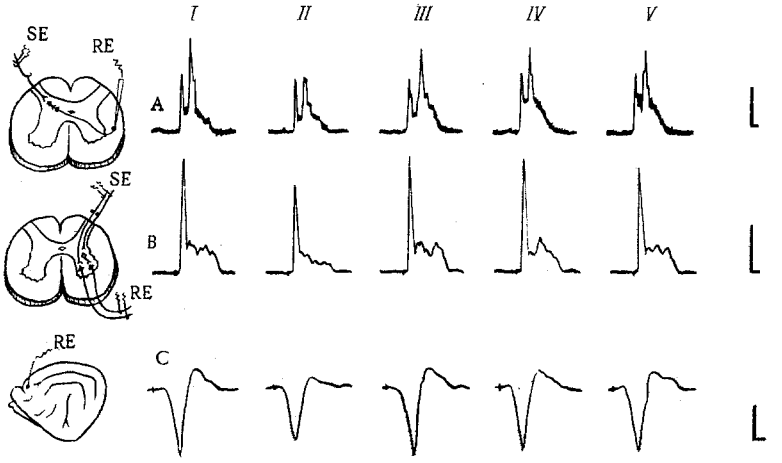

Fig. 1. Effect of leucine-enkephalin on EPs at different levels of the CNS to single sciatic nerve stimulation. A) EPs in ventrolateral columns of spinal cord; B) mono- and polysynaptic spinal cord potentials; C) EPs of cortical somatosensory area I. I) Background; II) 5 min after injection of leucine-enkephalin (1 mg) into lateral ventricle; III) recovery; IV) 10 min after injection of naloxone (1 mg/kg, intravenously); V) 5 min after second injection of leucine-enkephalin. Left: scheme showing arrangement of stimulating electrodes (SE) and recording electrodes (RE). Right: vertical lines, calibration of EP amplitude (A 50 μV, B 100 μV, C 100 μV); horizontal lines, time marker (A 5 msec, B 5 msec, C 20 msec).

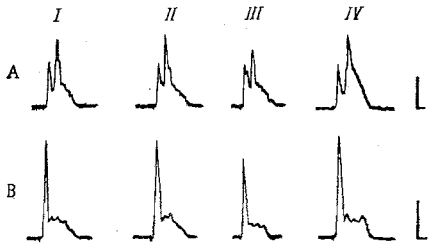

Fig. 2. Effect of leucine-enkephalin on EPs in spinal cord. A) EPs in ventrolateral columns of spinal cord; B) mono- and polysynaptic spinal cord potentials. I) Background; II) 45 min after injection of methysergide (2.5 mg/kg, intraperitoneally); III) 5 min after injection of leucine-enkephalin (1 mg) into lateral ventricle; IV) recovery of EP. Vertical lines: calibration of amplitude (A 50 μV, B 100 μV); horizontal lines: time marker (A and B, 5 msec).

motor branch of the mandibular nerve to supramaximal stimulation of the lingual nerve, also were evaluated. The experiments began 2-2.5 h after inhalation of ether ceased. EPs were recorded by a monopolar method in the cortex and ventrolateral columns of the spinal cord, and those of the glossomandibular and segmental reflexes were recorded by a bipolar method. Leucine-enkephalin (1 mg in 0.1 ml Ringer's solution) was injected from a microinjector into the lateral ventricle through a burr-hole in the skull (coordinates A 14.0, L 2.5, H +7.0 according to Snider and Niemer [12]) in the course of 45-60 sec. Repeated injection of Ringer's solution into the lateral ventricle in control experiments did not cause changes in EP recordable in the above-mentioned CNS formations. Naloxone (1 mg/kg) was injected intravenously 10 min before or 3-5 min after injection of leucine-encephalin, and methysergide (2.5 mg/kg) was injected intraperitoneally 45 min before leucine-enkephalin. In the course of each experiment the blood pressure in the femoral artery was recorded and the animals' body temperature was kept constant.

EXPERIMENTAL RESULTS

Leucine-enkephalin was shown to cause a decrease in amplitude of EP in the ventrolateral columns of the spinal cord by 30-50% ($P < 0.01$). A more marked decrease in the amplitude of EP was observed on the contralateral side relative to the stimulated nerve (Fig. 1A). This polypeptide also characteristically modified segmental synaptic transmission in the spinal cord (Fig. 1B): The amplitude of the monosynaptic response fell by 15-20% ($P < 0.01$) and that of the polysynaptic by 50-60% ($P < 0.01$). The maximal effect of the action of leucine-enkephalin was observed 5 min after injection of this compound, and after 15 min the amplitude of EP was at its initial level. Repeated injections of leucine-enkephalin (with intervals of 30 min between injections) were unaccompanied by any manifestations of tachyphylaxis. After preliminary (10 min before injection of leucine-enkephalin) intravenous injection of naloxone (1 mg/kg) no changes in EP were found in the ventrolateral columns of the spinal cord, or in the amplitude of the monosynaptic and polysynaptic spinal cord potentials (Fig. 1). Administration of naloxone 3-5 min after injection of leucine-enkephalin led to rapid recovery of the above responses.

Methysergide (2.5 mg/kg) caused no significant change in the amplitude of EPs to sciatic nerve stimulation in the ventrolateral columns, and also in the anterior roots of the spinal cord, but it reduced the inhibitory effect of leucine-enkephalin. For instance, injection of leucine-enkephalin 45-60 min after preliminary injection of methysergide led to only very slight changes (7-12%) in the amplitude of the evoked responses (Fig. 2).

Leucine-enkephalin did not change the amplitude of EPs arising in the motor branch of the mandibular nerve to stimulation of the lingual nerve, but changed the rhythm of brain electrical activity. Paroxysmal discharges were observed on the EEG.

Leucine-enkephalin reduced the amplitude of EP in somatosensory area I of the cortex evoked by single supramaximal stimulation of the sciatic nerve. Naloxone, which did not affect cortical EPs, completely prevented the inhibitory action of leucine-enkephalin (Fig. 1C).

This investigation thus showed that leucine-enkephalin inhibits interneuronal transmission in the ventrolateral columns of the spinal cord, modifies segmental monosynaptic and polysynaptic spinal cord potentials, does not affect the central components of the glossomandibular reflex, and slightly reduces EPs in cortical sensomotor area I. Similar changes in interneuronal transmission in the CNS have been observed following the use of preparations of the morphine group in curarized cats [1, 2, 10]. Baxter et al., [3] and Urca et al., [15] have obtained evidence of the ability of morphine and enkephalins to cause the appearance of paroxysmal discharges on the EEG following intraventricular injection of these compounds. The above-mentioned effects of leucine-enkephalin are distinguished by their specificity, for they are prevented or abolished by naloxone, an antagonist of narcotic analgesics [4, 5]. Methysergide, which blocks serotoninergic receptor structures, also diminished the effect of leucine-enkephalin on the spinal cord; this confirms the view that the influence of narcotic analgesics and of morphine-like polypeptides on the spinal cord is connected with their activation of descending inhibitory influences, for which, in the opinion of Hokflet et al. [6], serotonin is the possible mediator.

LITERATURE CITED

1. A. G. Rudakov, "Neuropharmacological investigation of fentanyl and droperidol," Author's Abstract of Candidate's Dissertation, Moscow (1972).
2. L. N. Sinitsyn, "The effect of neurotropic drugs on afferent and efferent visceral systems," Author's Abstract of Doctoral Dissertation, Moscow (1974).
3. M. G. Baxter, D. Goff, A. A. Miller, et al., Brit. J. Pharmacol., 59, 455 (1977).
4. J. D. Beluzzi, N. Grant, V. Garsky, et al., Nature, 260, 625 (1976).
5. H. H. Buscher, C. R. Hill, and D. Romer, Nature, 261, 423 (1976).
6. T. Hökfelt, A. Ljungdahl, L. Terenius, et al., Proc. Natl. Acad. Sci. USA, 74, 3081 (1977).
7. J. Hughes, T. W. Smith, and H. W. Kosterlitz, Nature, 258, 577 (1975).
8. K. Oliver and W. Funderburk, Electroenceph. Clin. Neurophysiol., 19, 501 (1965).
9. J. L. Oliveras, F. Redjemi, K. Guilbaud, et al., Pain, 1, 139 (1975).
10. M. Satoch and H. Takagi, Eur. J. Pharmacol., 14, 60 (1971).
11. R. Simantov and S. H. Snyder, Life Sci., 18, 781 (1976).
12. R. S. Snider and W. T. Niemer, A Stereotaxic Atlas of the Cat Brain, Chicago (1961).
13. L. Terenius, Acta Physiol. Scand., 98, Suppl., 5 (1976).
14. G. Urca, H. Erenk, and J. C. Liebeskind, Science, 197, 83 (1977).
15. M. Vogt, J. Physiol. (London), 236, 438 (1974).