MORPHINE-LIKE ACTIVITY OF THE ENKEPHALIN ANALOG Tyr-D-Ala-Gly-Phe-NH_2

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Endogenous morphine-like compounds methionine-enkephalin and leucine-enkephalin are known to produce short-term analgesia only if injected into the cerebral ventricles. Replacement of glycine in the second position by D-alanine and protection of the C-terminal by an amino group increased the analgesic activity and duration of action of the enkephalins because of their resistance to plasma and CSF enzymes. McGregor et al. [3] first reported the analgesic activity of an enkephalin-like tetrapeptide Tyr-D-Ala-Gly-Phe-NH_2. This compound has the property of binding with opiate receptors of the brain *in vitro* and also produces marked analgesia when injected by intraventricular and intravenous routes. In connection with the obtaining of an enkephalin analog active by intraveous injection, it was decided to study the effect of the tetrapeptide on interneuronal transmission of excitation in different parts of the CNS and also to investigate its analgesic activity and its effect on peripheral opiate receptors. The tetrapeptide used in the present investigation was obtained in the Laboratory of Peptide Synthesis (Head, Dr. Chem. Sci. M. I. Titov) of the All-Union Cardiologic Scientific Center.

EXPERIMENTAL METHOD

To study the effect of the tetrapeptide on interneuronal transmission of excitation in the CNS two methods were used: the method of impulse summation and the method of extracellular recording of spontaneous and evoked unit activity in the sensomotor cortex. Impulse summation was studied in experiments on intact rabbits. Extracellular sensomotor cortical unit activity was carried out on rats weighing 350-500 g. The methods used were described in detail by the writers previously [1]. Analgesic activity of the tetrapeptide and of morphine was compared in experiments on mice by Hafner's method, modified by Takagi [5]. The response to stimulation was evaluated on the alternative principle 5, 15, 30, 60, and 90 min after intravenous injection of the tetrapeptide or morphine. ED_{50} was calculated by the method of Litchfield and Wilcoxon on the basis of data obtained 15 min after injection of the preparation. Each dose of the preparation was tested on six to eight animals. The effect of the tetrapeptide on peripheral opiate receptors of the mouse vas deferens *in vitro* also was studied. The isolated vas was placed in a 20-ml beaker containing modified Krebs' solution (concentration of components in mM: NaCl 118, KCl 4.75, $CaCl_2$ 2.59, $NaHCO_3$ 25, KH_2PO_4 0.93, glucose 11), and aerated with a mixture of 95% oxygen and 5% CO_2. The vas was stimulated with single square stimuli of supramaximal amplitude, 20 msec in duration and with a frequency of 0.2 Hz, through ring electrodes. Contractions were recorded isometrically by means of a 6MKhIS mechanotron transducer on an N338 recorder. Each successive preparation was added after the vas had been rinsed for 15 min.

EXPERIMENTAL RESULTS

The experiments showed that the tetrapeptide inhibits impulse summation. For instance, initial depression of impulse summation was observed after intravenous injection of the tetrapeptide in a dose of 1 mg/kg: Injection of the preparation in smaller doses did not change the impulse summation. Marked inhibition of summation was observed after injection of the tetrapeptide in a dose of 2-2.5 mg/kg (Fig. 1). Its inhibitory action increased with an increase

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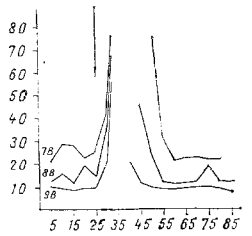

Fig. 1. Effect of tetrapeptide on impulse summation in rabbit CNS. Numbers near traces show amplitude of stimuli in V. Arrow indicates time of injection of tetrapeptide in dose of 2.5 mg/kg. Abscissa, time (in min); ordinate, number of stimuli.

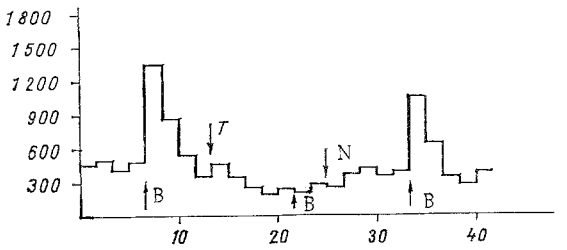

Fig. 2. Effect of tetrapeptide and naloxone on spontaneous and bradykinin-induced activity of rat sensomotor cortical neurons. Columns represent number of discharges during each 100 sec. B) intraarterial injection of bradykinin (10 μg); T) intravenous injection of tetrapeptide (2 mg/kg); N) intravenous injection of naloxone (1 mg/kg). Abscissa, time (in min); ordinate, number of discharges.

in the dose. Naloxone, an antagonist of the narcotic analgesics, if injected intravenously in a dose of 1 mg/kg, abolished the inhibitory effect of the tetrapeptide on impulse summation. Morphine is known to induce inhibition of impulse summation which is abolished by opiate antagonists. The tetrapeptide and morphine thus had a similar effect on impulse summation. However, in its ability to inhibit summation morphine is stronger than the tetrapeptide, for the analogous effect of morphine was found in doses half to two-thirds smaller than those of the tetrapeptide.

Investigation of the effect of the tetrapeptide on sensomotor cortical unit activity in rats showed that in a dose of 2 mg/kg intravenously the compound strongly inhibited discharges of the neurons (Fig. 2). The tetrapeptide inhibited spontaneous activity (by 40-60%) and also considerably weakened unit activity evoked by specific nociceptive stimulation during intraarterial injection of bradykinin [2]. With an increase in the load to 5 mg/kg the tetrapeptide suppressed spontaneous activity practically completely. Against the background of the tetrapeptide, bradykinin had no activating action on the sensomotor cortical neuron. In a dose of 1 mg/kg intravenously, naloxone completely abolished the effect of the tetrapeptide on unit activity.

A comparative study of the analgesic activity of the tetrapeptide and morphine by Takagi's method showed that ED_{50} for them was 9.3 (6.65-13.0) mg/kg and 6.3 (5.4-7.3) mg/kg, respectively. By this test the tetrapeptide was significantly weaker than morphine in its activity. The analgesic action of these substances in the above doses lasted about 30 min, but if they were given in doses close to ED_{95}, the duration of analgesia was increased to 60 min. Naloxone, if injected intraperitoneally beforehand in a dose of 2 mg/kg, completely prevented the development of analgesia induced by either the tetrapeptide or morphine.

In experiments to study the effect of the tetrapeptide and morphine on contractions of the mouse vas deferens, both were found to cause dose-dependent inhibition of contractions of the vas (Fig. 3). The concentration of morphine at which contractions of the vas were inhibited by 50% (EC_{50}) was $5.6 \cdot 10^{-6}$ ($1.3 \cdot 10^{-6}$ to $9.9 \cdot 10^{-6}$) M, whereas EC_{50} for the tetrapeptide was $4.9 \cdot 10^{-7}$ ($3.6 \cdot 10^{-7}$ to $6.2 \cdot 10^{-7}$) M. These differences in the activity of the prepa-

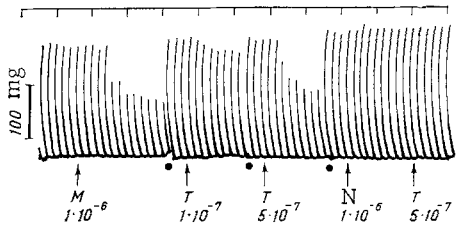

Fig. 3. Effect of morphine and tetrapeptide on contractions of isolated mouse vas deferens induced by electrical stimulation. Arrow indicates time of administration of substances, dots show time of rinsing preparation. M) morphine; T) tetrapeptide; N) naloxone. Molar concentrations of substances are shown.

rations are statistically significant ($P = 0.05$). Naloxone in a concentration of $1 \cdot 10^{-6}$ M abolished the inhibitory action of both morphine and the tetrapeptide. As regards their effect on contractions of the vas deferens, the tetrapeptide was therefore more active than morphine.

The effects of narcotic analgesics and of morphine-like peptides are known to be connected with their influence on what are called opiate receptors [4]. The analgesic action of the tetrapeptide and morphine observed in the present investigation and their inhibitory effect on impulse summation, on sensomotor cortical unit activity, and on contractions of the vas deferens are due to their interaction with opiate receptors located in the central and peripheral nervous system. This conclusion is based on the fact that all effects of the tetrapeptide and morphine observed in the present investigation were blocked by naloxone, a specific antagonist of the opiate.

These results are evidence that the tetrapeptide and morphine differ in their effect on opiate receptors in different situations. Morphine has a stronger action on opiate receptors in the CNS whereas the tetrapeptide has a stronger action on opiate receptors of the vas deferens.

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