ACTION OF KYOTORPHIN ON HIPPOCAMPAL NEURONAL REACTIVITY

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In 1979 the dipeptide Tyr-Arg (kyotorphin) was isolated from brain tissue and identified; on intracisternal injection it possesses higher analgesic activity than the enkephalins [11, 13]. Investigation of the possible mechanisms of its action showed that it binds weakly with opiate receptors, but increases the release of endogenous enkephalins by several times [8, 12, 13].

Data on the distribution of kyotorphin in the brain indicate that the functional role of this peptide is most probably not confined to its participation in the pain inhibition system: Although the kyotorphin content is quite high in regions connected with pain sensitivity, it is present also in other structures, and about 50% of the total brain kyotorphin is concentrated in the neocortex [15].

Despite the marked central action of kyotorphin and its presence in several brain formations, the effect of the pepetide on brain neuronal activity has virtually not been studied. The hippocampus, the central structure of the brain limbic system, contains both kyotorphin [15] and also a large number of enkephalinergic cells and opiate receptors [5, 6, 9]. It was therefore interesting to study the action of kyotorphin on hippocampal neuronal reactivity and to assess the role of enkephalins and opiate receptors in the mechanisms of this action.

The concrete aims of the investigation were as follows: to study the action of kyotorphin on reactivity of pyramidal neurons in areas CA1 and CA3 during stimulation of Schaffer's sollaterals and mossy fibers respectively, to compare effects of kyotorphin with the action of Met-enkephalin, and to investigate the effect of naloxone, which blocks opiate receptors, on them. Experiments were carried out on hippocampal slices, which are a convenient and adequate model for electrophysiological and pharmacological research.

EXPERIMENTAL METHODS

Transverse slices of the hippocampus were prepared from the brain of Wistar rats aged 3-6 weeks, and transferred into a continuous-flow chamber filled with medium oxygenated and warmed to 31-33°C, and fixed on a nylon grid, located 2 mm below the level of the liquid. A modified Ringer's solution of the following composition (in mM) was used as the medium: NaCl 124, KCl 3, $CaCl_2$ 2.5, $MgSO_4$ 2.4, KH_2PO_4 1.25, $NaHCO_3$ 26, D-glucose 10, pH 7.2-7.4. Before use the solution was saturated with a gas mixture of 95% O_2 + 5% CO_2. The flow rate was 1.5-2 ml/min.

Electrical activity began to be recorded 1.5-2 h after preparation of the slice. The recording microelectrode, filled with 3M NaCl solution, was introduced into the pyramidal layer of area CA1 or CA3. Stimulating bipolar electrodes, made from glass capillary tubes and filled with perfusion solution, were applied to the radial layer to activate Schaffer's collaterals or to the granular layer and hilus of the dentate fascia to activate mossy fibers.

Field potentials evoked by single periodic stimulation (square pulse, 10-15 V, 0.1 msec, 0.1 Hz) of the corresponding synaptic input were recorded and analyzed with a PDP 8a minicompouter (USA). The action of the substances on reactivity was assessed relative to

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TABLE 1. Changes in Reactivity of Hippocampal Pyramidal Neurons under the Influence of Kyotorphin

Effect	CA1	CA3
Facilitation	7	5
Inhibition	3	2
No change	4	2

Note. Data given (number of preparations) for first addition of substance in concentrations of 1-10 μM in experiments on area CA1 and in concentration of 10-100 μM in experiments on area CA3.

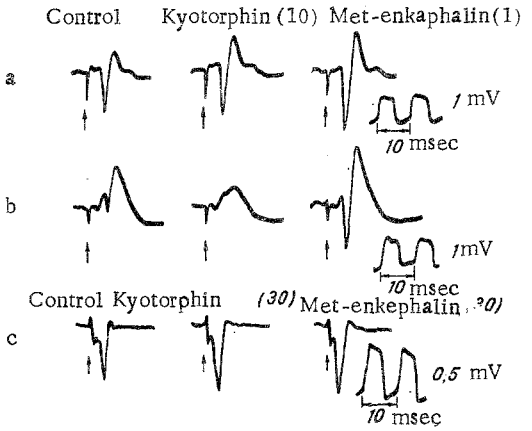

Fig. 1. Comparison of action of kyotorphin and Met-enkephalin on field potentials recorded in areas CA1 and CA3 in response to stimulation of Schaffer's collaterals and mossy fibers respectively. A, B, C) Different experiments. A, B) Recording from Ca1; C) from CA3. Averaging of 20 responses (stimulus indicated by arrow) before and during perfusion of preparation with solution containing substance (4-6 min after beginning of perfusion). Here and in Fig. 2: concentration of substance shown in parentheses (in μM).

current amplitude of the population spike reflecting the synchronized discharge of a pyramidal cell population [3], and also from averaged responses for 20 realizations.

The substances were dissolved in perfusion medium and introduced into the chamber by switching the flow system to the appropriate reservoir. Kyotorphin was synthesized by the method of activated esters, using unprotected arginine in 50% dioxan [1]. The concentration of the basic substance, according to the data of liquid chromatography on the LS-2000 amino-acid analyzer (Biotronic, West Germany, DC-1A resin), was 96%. Met-enkephalin, synthesized in the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, and naloxone (Endo Laboratories, USA) also were used.

RESULTS

In most preparations (Table 1) administration of kyotorphin caused facilitation of reactivity of the pyramidal neurons, manifested as an increase in amplitude of the population spike. The degree of facilitation in area CA1 in response to addition of 5 μM of the peptide (five slices) was 36 ± 17%, whereas the degree of facilitation in area CA3 in response to 50 μM of the peptide (four slices) was 49 ± 18%.

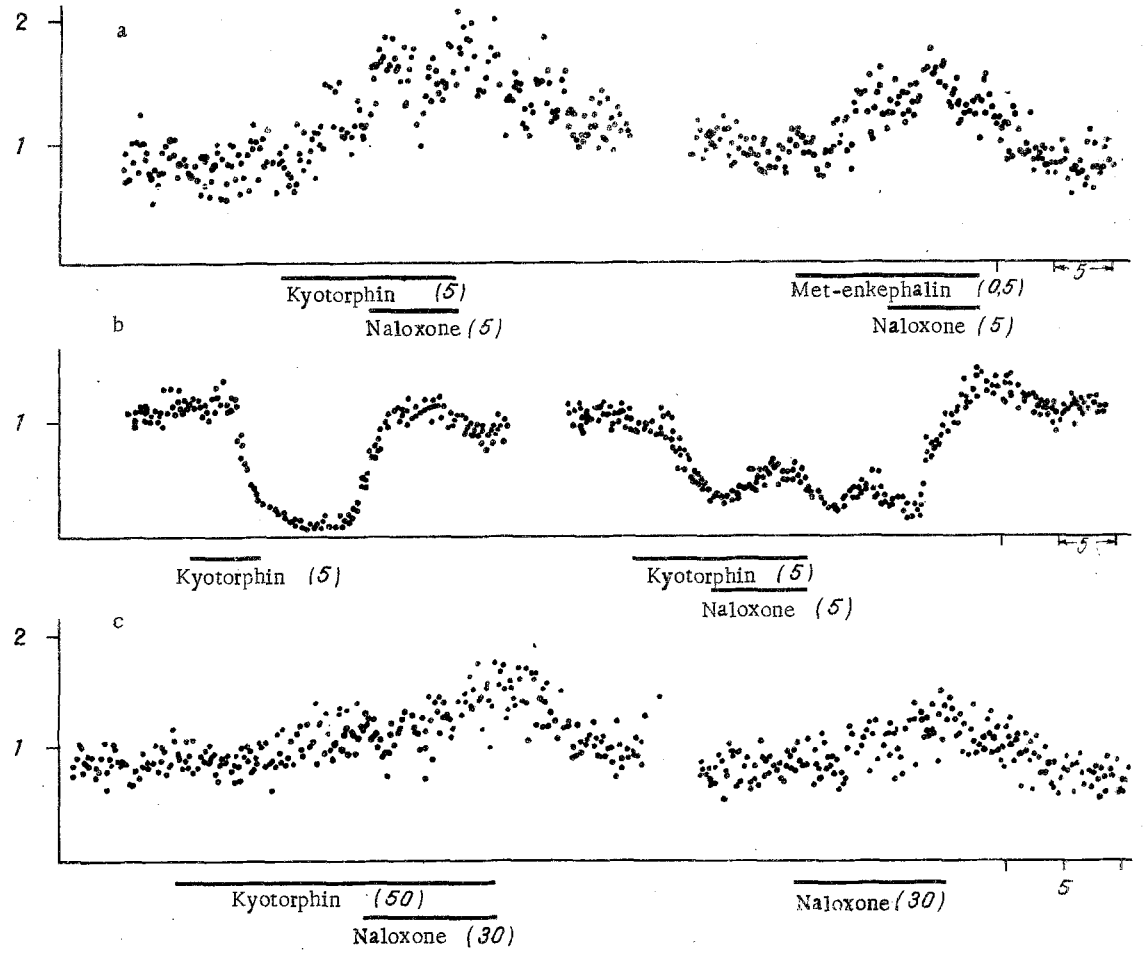

Fig. 2. Effect of naloxone on changes in reactivity induced by kyotorphin. A, B, C) Graphs of current amplitude of population spike in three different experiments (A and B on area CA1; C on area CA3). Abscissa, time (in min); ordinate, amplitude of population spike measured from minimum to maximum of spike wave of field potential (in mV). Time of perfusion indicated by straight line.

Dependence of the magnitude of the effect on the concentration of the substance could not be studied systematically because, if a response to the first addition of the substance occurred, subsequent tests, even with increasing concentrations, did not always prove effective: The action of not more than two or three additions could be tested on one preparation. There was also great scatter of the individual sensitivity of the sections. On the whole, acting concentrations of the substance (the criterion of action was a change in magnitude of the focal response by not less than 20% relative to the initial level) were between 1 and 10 μM when recorded in area CA1, and an order of magnitude higher in the experiments on area CA3.

In concentrations inducing a two-fourfold increase in spontaneous release of endogeneous Met-enkephalin, and in secretion by slices of the striatum evoked by electrical stimulation [10, 12], kyotorphin thus has a well-marked and mainly facilitating action on reactivity of hippocampal pyramidal neurons. It can be tentatively suggested that the mechanism of action of kyotorphin in the hippocampus is intensification of release of endogenous enkephalines and their reception by the corresponding structures. This is shown by the similarity of the active concentrations in the present experiments and in those on sections through the striatum [8, 10, 12], and also the ineffectiveness of repeated additions of the substance, which may have been due to exhaustion of enkephalin reserves in the terminals.

To assess the possible role of enkephalins and opiate receptors in the mechanisms of action of kyotorphin, changes in reactivity induced by kyotorphin and Met-enkephalin and the sensitivity of these effects to naloxone were compared in the same preparations. This

comparison was done chiefly in area CA1, because addition of extrinsic enkephalins in concentrations of 10^{-6}-10^{-5} M had virtually no effect on field potentials recorded in area CA3 in response to stimulation of mossy fibers [2].

In all 10 experiments on CA1 addition of 0.5-5 μM Met-enkephalin caused a two-to threefold increase in the amplitude of the focal response (Fig. 1a, b). In most cases (7 of 10 experiments) kyotorphin also caused facilitation (less marked), but in three experiments reactivity was inhibited. Examples of kyotorphin and Met-enkephalin acting in the same and opposite directions are given in Fig. 1.

Naloxone, in concentrations of between 1 and 10 μM completely or partly abolished the facilitation induced by the enkephalin (Fig. 2a) but did not affect facilitation induced by kyotorphin (Fig. 2a, b). Thus, despite the external similarity of the effects, they are most probably mediated by different mechanisms, although it can be postulated that endogenous enkephalins interact with different receptors from those which interact with extrinsic enkephalins, and that these receptors are insensitive to naloxone. Receptors of this type (σ) have recently been described in the hippocampus, where they are only half as numerous as μ-receptors, but about four times more numerous than δ-receptors [14].

Inhibition of reactivity by kyotorphin likewise was not abolished by naloxone, although in two of three experiments addition of the antagonist prevented the full development of the effect (Fig. 2b). However, this counteraction may perhaps be associated with the ability of naloxone to induced facilitation of responses in some preparations (Fig. 2c), probably due to its interaction with the GABA-ergic inhibitory system [4, 7].

The investigation thus showed that the dipeptide kyotorphin has a marked action on reactivity of hippocampal neurons. The question whether this action is linked with release of endogenous enkephalins or whether it is due to certain other mechanisms cannot yet be answered, and additional neurochemical and electrophysiological investigations are necessary before this can be done.

LITERATURE CITED

1. A. B. Virovets, K. S. Martynov, and M. I. Titov, Zh. Obshch. Khim., 38, 2337 (1968).
2. A. N. Chepkova, Byull. Éksp. Biol. Med., No. 5, 6 (1983).
3. P. Andersen, T. V. P. Bliss, and K. K. Strede, Exp. Brain Res., 13, 208 (1971).
4. R. Dingledine, L. L. Iversen, and E. Breuker, Europ. J. Pharmacol., 47, 19 (1978).
5. T. Duka, M. Wuster, P. Schubert, et al., Brain Res., 205, 181 (1980).
6. C. Gall, N. Brecha, H. J. Karten, and K. J. Chang, J. Comp. Neurol., 198, 335 (1981).
7. D. L. Gruol, J. L. Barker, and T. G. Smith, Brain Res., 198, 323 (1980).
8. P. K. Janicki and A. W. Lipkowski, Neuosci. Lett., 43, 73 (1983).
9. R. C. Meiback and S. Maayani, Europ. J. Pharmacol., 68, 175 (1980).
10. H. Shiomi, Y. Kuraishi, H. Ueda, et al., Brain Res., 211, 161 (1981).
11. H. Shiomi, H. Ueda, and H. Takagi, Neutopharmacology, 20, 633 (1981).
12. H. Takagi, H. Shiomi, Y. Kuraishi, and H. Ueda, Experientia, 38, 1344 (1982).
13. H. Takagi, H. Shiomi, H. Ueda, and H. Amano, Nature, 282, 410 (1979).
14. S. W. Tam, Proc. Nat. Acad. Sci. USA, 80, 6703 (1983).
15. H. Ueda, H. Shiomi, and H. Takagi, Brain Res., 198, 460 (1980).