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OPIOID PEPTIDES AS REGULATORS OF ACETYLCHOLINESTERASE ACTIVITY

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UDC 615.31:547.95:547.943].015.4:
[616.154:577.175.822]-088.931

KEY WORDS: acetylcholinesterase; endorphins; enkephalins; regulation of enzyme activity.

Because peptides are present in synaptic endings of neurons along with "classical" neurotransmitters [9] and since these are released together during depolarization of the axon membrane, it has been claimed that the modulating role of peptides can be reduced either to modification of the characteristics of excitable membranes or to their action on the release, reception, and metabolism of a mediator [5]. On the basis of a certain analogy between receptor and enzyme, we postulated that peptide hormones can act directly on the enzymes of neurotransmitter metabolism.

The aim of this investigation was to study the direct action of opioid peptides, including enkephalins and their fragments and short endorphins, on activity of acetylcholinesterase (AChE; EC 3.1.1.7), the enzyme hydrolyzing the cholinergic neurotransmitter acetylcholine.

EXPERIMENTAL METHOD

A water-soluble preparation of AChE from human blood erythrocytes [1] (Research Institute of Vaccines and Sera, Perm'), identical to the brain enzyme for substrate-inhibitor specificity [7], was used as the model with which to study peptide interaction. Preparations of peptide hormones were generously provided by M. I. Titov (All-Union Cardiologic Scientific Center, Ministry of Health of the USSR, Moscow). AChE activity was determined by a modified Ellman's method [8] at 37°C (pH 7.5), using acetylthiocholine bromide as the

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(Presented by Academician of the Academy of Medical Sciences of the USSR I. P. Ashmarin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 10, pp. 457-459, October, 1989. Original article submitted February 20, 1989.

0007-4888/89/0010-1451 $12.50

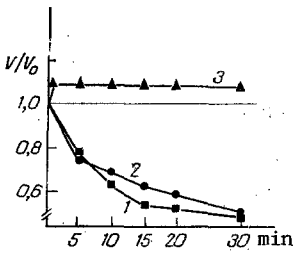

Fig. 1

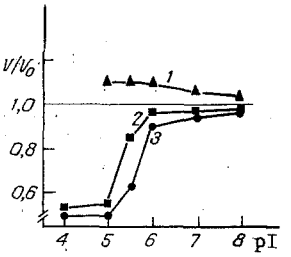

Fig. 2

Fig. 1. Dependence of effector action of opioid peptides in a concentration of 10^{-5} M on AChE activity on duration of preincubation of peptides with enzyme in absence of substrate. 1) Met-enkephalin; 2) Leu-enkephalin; 3) α-endorphin.

Fig. 2. Dependence of effector action of opioid peptides on AChE on their concentration. 1) α-Endorphin; 2) Met-enkephalin; 3) Leu-enkephalin; pI) negative logarithm of peptide concentration (duration of preincubation of peptide with enzyme 30 min).

substrate. Values of maximal reaction velocity (V) and Michaelis' constant (K_m) were calculated on a linear graph [6]. The effectiveness of action of the peptide ligands was judged by values of the activation constant (K_A) and the generalized inhibitor constant $\overline{K}_i$ for reversibly acting effectors. When calculating K_i it was assumed that the value of this constant is the sum of its competitive K_i and noncompetitive K'_i components, in accordance with the formula: $1/\overline{K}_i + 1/K_i + 1/K'_i$ [3, 4]. To determine the values of these constants we measured the initial velocities of enzymic hydrolysis in experiments in the absence and in the presence of inhibitors, calculated the values of K_i and K'_i by an analytical method, and expressed the values obtained as negative logarithms to base 10: $p\overline{K}_i$, pK_i, and pK'_i. The type of inhibition was determined from the relationship between the values of pK_i and pK'_i, namely: mixed $pK_i > pK'_i$, competitive $pK'_i \to 0$ and $p\overline{K}_i = pK_i$, noncompetitive $pK_i = pK'_i$. The value of K_A was calculated analytically by the method suggested previously [2] and expressed in the form pK_A. The type of activating action was determined from the relationship between the numerical values of the coefficients of the equation for the initial velocity of the enzyme reaction α and β [2]. For instance, during mixed activation $\alpha \neq 1$ and $\beta \neq 1$, for synergistic activation $\alpha < 1$, $\beta = 1$, uncompetitive $\alpha = \beta > 1$, and noncompetitive activation $\alpha = 1$, $\beta > 1$. The value of the effect was expressed as the ratio between velocities of the enzyme reaction in the presence and absence of the inhibitor (V/V_0).

EXPERIMENTAL RESULTS

Preliminary experiments showed that enkephalins and their fragments are AChE inhibitors, whereas short endorphins have an activating action on the enzyme.

The study of the inhibitory action of enkephalins and their fragments showed that their influence on AChE increased with an increase in the duration of preincubation of peptides with enzyme at 37°C in the absence of the substrate (Fig. 1), but this is evidence in support of possible conformational changes in the enzyme molecule rather than of the irreversible character of action of the inhibitor, since an increase in the concentration of buffer or a change in pH (data not given) completely abolished the effect. Activity of the enzyme remained constant during preincubation. Evidence of stability of the peptides in the incubation medium was given by direct radioimmunoassay of Met-enkephalin (data not given). The activating action of α- and γ-endorphins was independent of the duration of preincubation of these compounds with the enzyme. Thus the opioid peptides which we studied proved to be reversible effectors relative to AChE.

As Table 1 shows, enkephalins and some of their fragments were reversible inhibitors of AChE with a noncompetitive type of action, as shown by the commensurate values of pK_i and pK'_i, and this suggests adsorption of the peptides both on the active center of the enzyme and outside it.

Analysis of values of $p\overline{K}_i$ of enkephalins (I-II), the analog III, and their fragments (IV-XIII) during the study of the action of these compounds on AChE showed that the specific

TABLE 1. Cholinesterase Effectiveness of Enkephalins, their Fragments, and Short Endorphins ($p\overline{K}_i$, pK_i, pK'_i, pK_A denote negative logarithms of generalized, competitive, and uncompetitive inhibition constants and activation constant. α and β denote coefficients of equation of initial velocity of enzyme reaction)

Peptide	Effect	$p\overline{K}_t$	pK_i	pK'_i
I Tyr—Gly—Cly—Phe—Leu	I	5,40	5,10	5,10
II Tyr—Gly—Gly—Phe—Met	I	5,24	4,94	4,93
III Tyr—DAla—Gly—Phe—DLeu	I	5,15	4,87	4,82
IV Tyr—Gly—Gly—Phe	I	3,90	3,59	3,59
V Tyr—DAla—Gly—Phe	I	4,21	3,91	3,91
VI Tyr—Dala—Gly	I	3,95	3,65	3,65
VII Tyr—DAla	O	<3,00		
VIII DAla—Gly—Phe—Leu	O	<3,00		
IX DAla—Gly—Phe	I	4,01	3,71	3,71
X DAla—Gly	I	3,63	3,33	3,33
XI Gly—Phe—Leu	O	<3,00		
XII Gly—Phe	O	<3,00		
XIII Phe—Leu	I	3,84	3,54	3,54
		pK_A	α	β
XIV Tyr—Gly—Gly—Phe—Met Thr—Ser—Glu—Lys—Ser Gln—Thr—Pro—Leu—Val—Thr	A	7,60	1,00	1,09
XV Tyr—Ghy—Gly—Phe—Met Thr—Ser—Glu—Lys—Ser Gln—Thr—Pro—Leu—Val—Thr—Leu	A	7,03	1,00	1,08

Legend. I) Inhibition, A) activation, 0) no effect within range of concentrations tested (10^{-8}-10^{-4} M, $p < 0.001$).

amino-acid sequence necessary for the maximal inhibitory effect among the series of compounds tested is the amino-acid sequence observed in natural pentapeptides, for enkephalins possessed the greatest inhibitory activity, and loss of the C-terminal amino acid and subsequent shortening of the molecule led to a sharp decrease in magnitude of the effect or to its total loss.

The study of the action of α- and γ-endorphins (fragments 67-76 and 61-77 of β-lipotrophin), the N-terminal amino-acid sequence of which is Met-enkephalin, on AChE showed that these peptides are noncompetitive activators of the enzyme and have closely similar values of pK_A (XIV-XV). Dependence of the effector action of these peptide hormones on AChE on their concentration is shown in Fig. 2. An S-shaped curve of dependence of the effect on the enzyme on peptide concentration is characteristic of all these compounds.

The noncompetitive type of both inhibitory and activating action and the S-shaped curve of dependence of effect on concentration of opioid peptides, and also dependence of the inhibitory action of enkephalins on AChE on the duration of preincubation lead to the conclusion that opioid peptides are allosteric regulators of AChE.

Regulation of AChE by endorphins and enkephalins, which are products of successive proteolysis of β-lipotrophin, is thus an example of the existence of regulation of enzyme activity by a direct (not indirectly through the receptor) method and of the uniting of a set of bioregulators of AChE in one protopeptide. Consequently, through their action on AChE activity, opioid peptides can exert a modulating influence on processes connected with the action of acetylcholine.

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MODIFICATION OF SELECTIVE ADSORPTION OF MUSCARINIC ANTAGONISTS ON BRAIN MEMBRANES DURING CHLOROPHOS* POISONING

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UDC 615.217.34.03:[616-099-02:615.285.7].015.4:612.82.2].076.9

KEY WORDS: specific binding of cholinolytics; chlorophos; dipyroxime; rat brain.

The traditional method of treatment of poisoning by organophosphorus pesticides, chlorophos* in particular, is by the combined use of cholinolytics and cholinesterase reactivators, in order to protect the acetylcholine (ACh) receptor against the action of an excess of ACh and to restore activity of inhibited cholinesterase. The pharmacologic effects of cholinolytics, due to ACh receptor blockade, have now been confirmed at the level of specific binding with membranes of the brain and peripheral organs [4-7]. It has also been shown that certain cholinesterase reactivators bind selectively with ACh receptors [2, 3, 9]. Accordingly, the phenomenon of potentiation of therapeutic effects observed during combined treatment with cholinolytics and reactivators [1] could be the result of their interaction at the ACh receptor level.

The aim of this investigation was to study selective adsorption of muscarinic antagonists on rat brain membranes during chlorophos poisoning and the effect of the cholinesterase reactivator dipyroxime (trimedoxime bromide) on this process.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 130-160 g. To study binding of radioligands the brain of the decapitated animals, without the cerebellum, was homogenized in a glass homogenizer with Teflon pestle (11,000 rpm, 10 passages) in an ice-cold solution containing 10 mM Tris-HCl buffer (pH 7.4) and 1 mM EDTA. The homogenate (0.1%) was centrifuged for 20 min at 50,000 g. The residue was resuspended by rehomogenization in the same volume of solution containing 20 mM Tris-HCl buffer (pH 7.4) and 0.14 M NaCl, and centrifuged again under the above conditions. The residue was resuspended in twice the volume of the same buffer and used in experiments to study radioligand binding. Protein was determined by Lowry's method [8]. Aliquots of homogenate were incubated with increasing concentrations (0.02-6.0 nM) of ^{3}H-quinuclidinyl benzoate (^{3}H-QB; 1.11 TBq/mmole, from "Amersham," England), or with ^{14}C-cyclosyl (1.67 TBq/mole, State Institute of Applied Chemistry, Leningrad), in concentrations of between 0.5 and 60 mM. Binding of the radioligand inhibited by 10^{-5} M unlabeled atropine was regarded as specific. Nonspecific binding was 10% in the experiments with ^{3}H-QB and about 40% in those with cyclosyl. The samples were incubated for 30 min at 0°C and the reaction was stopped by

*O,O-dimethyl-1-hydroxy-2,2,2-trichloroethylphosphonate.

Institute of Toxicology, Ministry of Health of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR S. N. Golikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 10, pp. 459-461, October, 1989. Original article submitted February 20, 1988.

 0007-4888/89/0010-1454 $12.50