EFFECT OF THE AMINOPEPTIDASE INHIBITOR BESTATIN ON RAT BRAIN ENKEPHALIN LEVELS

N. A. Belyaev, E. F. Kolesanova, L. F. Kelesheva, T. V. Rotanova, and L. F. Panchenko

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There is now no shortage of experimental data to show that enkephalins control certain behavioral and emotional responses, endocrine functions of the pituitary gland, and various visceral reflexes, and that they may be involved in the pathogenesis of mental diseases and drugs additions [1, 3, 5, 9]. The study of the metabolism of these neuropeptides and ways of regulating it is therefore particularly important. One aspect of this problem is the study of enzymic degradation of enkephalins. In the existing view, inactivation of endogenous enkephalins in the brain can take place under the influence of two peptide hydrolases: an endogenous enzyme (enkephalinase A) and an aminopeptidase similar in its physicochemical properties to renal aminopeptidase M [6, 8, 13-15]. Involvement of enkephalinase A in enkephalinase A in enkephalin catabolism has been proved by several experiments [6, 13, 14], including during the study of the action of thiorphan, a selective inhibitor of this enzyme, on the Met-enkephalin concentration in brain tissue [15]. The possibility of inactivating these peptides by an aminopeptidase-M-like enzyme has been confirmed by a mass of indirect evidence [4, 6-8]. Direct confirmation could be obtained by determining changes in brain enkephalin levels under the influence of bestatin, an effective aminopeptidase inhibitor [12].

EXPERIMENTAL METHOD

Experiments were carried out on 44 noninbred male albino rats weighing 250-300 g, into whose lateral ventricles cannulas were implanted 3-4 days before the experiment began. Rats of the experimental group received bestatin in a dose of 200 μg per animal, via the cannula, in the form of a solution in dimethylsulfoxide. Animals of the control group received the equivalent volume (5 μl) of dimethylsulfoxide.

The animals were decapitated. The midbrain (including the hypothalamus) and corpus striatum after isolation were frozen on a metal plate, cooled in dry ice, and kept until use at −80°C.

Peptide material was extracted from the brain tissues in 1 N acetic acid, as described previously [2].

The enkephalin concentrations in the samples were determined by radioimmunoassay, using kits from "Incstar" (USA).

Bestatin [3(R)-amino-2(RS)-oxy-4-phenylbutanoyl-L-leucine] was synthesized by the method described previously [11]. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Table 1 gives data on the dynamics of Met-enkephalin and Leu-enkephalin levels in the striatum and midbrain (including the hypothalamus) of the rats after injection of bestatin. Microinjection of the inhibitor clearly affected the Met-enkephalin level in the midbrain (including the hypothalamus), and the effect lasted at least 24 h. Under these circumstances the peptide level fluctuated depending on the time after injection of the compound: it was raised 20 min after injection of bestatin, after 1 h it was at the control level, after 2 h

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TABLE 1. Concentrations of Enkephalins (in pg/mg tissue) in Striatum and Midbrain (including hypothalamus) of Rats after Administration of Bestatin (M ± m)

Part of brain	Time after injection	Met-enkephalin			Leu-enkephalin		
		control	experiment	% of control	control	experiment	% of control
Midbrain with hypothalamus	20 min	212±11	261±13*	123	115±13	138±18	120
	1 h	207±32	203±16	98	131±11	122±17	93
	2 h	225±8	181±11*	80	75,5±2,2	79,2±12	106
	24 h	206±33	288±48**	140	99,5±6,5	115±17	115
Striatum	20 min	600±51	535±53	89	87,6±6,7	72,2±7,2	82
	1 h	495±48	535±16	108	66,7±4,9	71,3±6,3	107
	2 h	454±50	516±46	114	96,7±26,6	79,3±12,4	82
	24 min	501±35	510±28	102	80,1±4,3	70,9±12,7	89

Note. *$p < 0.05$ compared with control; **$p < 0.05$ compared with experimental group "2 h," number of animals in each of experimental groups five or six.

it was depressed, and after 24 h it again exceeded the control level.

Accumulation of Met-enkephalin, observed in the brain tissue 20 min after injection of bestatin (against the background of analgesia induced by the drug, data not given) is evidence of slowing of enzymic hydrolysis of the peptide in situ. This result is in good agreement with data in the literature on the ability of bestatin to increase the release of Met-enkephalin from brain slices, induced by depolarization, and to cause analgesia, which is mediated by opioid peptides [4, 6]. Data contained in recently published works from Schwartz' laboratory prove that the efficacy of bestatin in experiments with brain slices is due to inbibition of a membrane-bound aminopeptidase, whose catalytic characteristics, sensitivity to inhibitors, and antigenic propertis are identical with those of the renal aminopeptidase M [7, 8]. Consequently, accumulation of Met-enkephalin in brain tissue, which we recorded after microinjection of bestatin, can be regarded as direct confirmation of the hypothesis that an aminopeptidase-M-like enzyme is involved in the inactivation of endogenous enkephalins in the brain. We know that bestatin has high metabolic stability and a long (about 24 h) half-elimination time from rats [10]. It can be tentatively suggested that the subsequent fall of the peptide level in the midbrain (including the hypothalamus) initially to the control level, and later substantially lower than this value, is due not to a decrease in the concentration of the inhibitor, but to the ability of the system for biosynthesis and release of Met-enkephalin in this region of the brain to adapt itself rapidly to changes in concentration of the peptide in situ. Evidence in support of this hypothesis is the fact that the Met-enkephalin level rose again 24 h after injection of the inhibitor into the animals' brain, which evidently caused adaptive changes in the rate of biosynthesis of the peptide, aimed at equalizing its steady-state concentration in the synaptic space. An alternative mechanism of this increase cannot be ruled out, namely due to decomposition of the adaptive inhibition of Met-enkephalin biosynthesis associated with continuing inhibition of aminopeptidase.

It is important to note that bestatin, which controls the Met-enkephalin level in the midbrain (including the hypothalamus), has not effect on the concentration of this peptide in the striatum, nor on the Leu-enkephalin concentration in both parts of the brain studied (Table 1). Considering that, besides the aminopeptidase, enkephalinase A also is involved in catabolism of the enkephalins [6, 13-15], the selectivity of action of bestatin thus revealed may indicate the presence of regional differences of Met-enkephalin catabolism in the brain and also differences in the contribution of aminopeptidase to the inactivation of Met-enkephalin and Leu-enkephalin. Future research will be aimed at solving these problems.

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EFFECT OF ASCORBIC ACID ON DECOMPOSITION OF ARACHIDONATE-15-HYDROPEROXIDE IN THE PRESENCE OF IRON SALTS AND COMPLEXES

D. A. Stoyanovskii, V. E. Kagan, and I. B. Afanas'ev

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A central role in the initiation and development of free-radical lipid peroxidation (LPO) reactions in vivo is played by ions of metals of variable valency and their complexes with intracellular ligands [8, 12]. It has been suggested that metals of variable valency participate in Fenton's reaction (initiation of LPO) and also in reactions of decomposition of hydroperoxides of polyenic fatty acids (chains lengthening reactions) [3]. In both types of reactions, the reduced states of metals with variable valency are active, and electron donors are needed to maintain them [4]. Components of electron-transport chains (enzyme systems of LPO induction), and also low-molecular-weight reducing agents (nonenzymic LPO) may act in the latter role in the cell. One such universal reducing agent, responsible for induction of LPO in vivo and also widely used in model system in vitro, is ascorbate [4]. However, high ascorbate concentrations have an inhibitory action on LPO, and the mechanisms of this effect have not been adequately studied. It is considered that ascorbate, in high concentrations, behaves as a trap for radicals, leading the oxidation chain [11]. However, the character of dependence of LPO inhibition by ascorbate on the Fe^{2+} concentration cannot be explained on the basis of this mechanism [2, 5, 14]. Other hypotheses have therefore been put forward recently to explain the mechanism of inhibition of LPO by high ascorbate concentrations [1].

The aim of the present investigation was to study the effect of ascorbic acid on decomposition of hydroperoxides of polyenic fatty acids (of 15-hydroperoxyarachidonate, 15-HPA) in the presence of iron and some of its complexes.

EXPERIMENTAL METHOD

15-HPA was synthesized by incubating arachidonic acid with soy lipoxygenase in 10 mM phosphate buffer, containing 0.2% deoxycholate (pH 7.4 at 25°C). The 15-HPA was purified from unreacted arachidonic acid and by-products of the reaction by extraction with chloroform followed by distributive extraction in a two-phase system containing a mixture of methanol: petroleum benzin:water in the ratio of 3:1:1. The formation and decomposition of 15-HPA were recorded spectrophotometrically on the basis of the characteristic absorption maximum of the conjugated dienes (monohydroperoxides) in the UV-spectrum at 234 nm [1] on a "Perkin-Elmer 552" spectrophotometer. In the experiments with ascorbic acid, 10% less ascorbic acid (50% less in the presence of EDTA) was added to the comparison cuvette than into the measuring cuvette. The ascorbate concentration at the end of the reaction was determined from the optical density at 264 nm [10] in the comparison cuvette.

The following reagents were used: ascorbate (from "Reanal," Hungary), soy lipoxygenase, deoxycholic acid, and arachidonic acid (from "Sigma," USA), and Na_2HPO_4, NaOH, EDTA, and

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