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Structural Requirements and Species Specificity of the Inhibition by β-Endorphin of Heavy Acetylcholinesterase from Vertebrate Skeletal Muscle

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SUMMARY

The molecular forms of acetylcholinesterase in extracts of gastrocnemius muscle from four vertebrate species and in electric eel (*Electrophorus*) electric organ were separated and identified by low-salt precipitation and velocity sedimentation. The activity of the heavy insoluble (A12) form of human muscle acetylcholinesterase was inhibited by synthetic human β-endorphin (500 mM). The homologous form in rat muscle extracts was poorly inhibited by human β-endorphin at the same concentration, but was more effectively inhibited by camel β-endorphin. The activities of heavy forms of pseudocholinesterase, present in small amounts in both species, were not reduced by β-endorphin. Selective inhibition of homologous heavy forms of acetylcholinesterase activity by camel and human β-endorphin was also seen in skeletal muscle extracts from frog and pigeon, but with decreased effectiveness. No inhibition was detectable in the heavy acetylcholinesterase form from extracts of electric organ tissue of the electric eel. The inhibition of heavy acetylcholinesterase activity in human muscle by human β-endorphin was dependent on the presence of its NH_2-terminal pentapeptide sequence. Maximal inhibitory potency depended on the presence of the entire amino acid sequence, since potency was considerably reduced in synthetic peptide analogues lacking either middle or COOH-terminal segments of β-endorphin. The relative potency of β-endorphin from various species as inhibitors of rat heavy acetylcholinesterase activity was also investigated. β-Endorphin sequences most closely resembling that of the rat peptide (camel, equine) were most potent, whereas those with sequence differences of more than one amino-acid were less potent (turkey, human) or had no inhibitory activity (ostrich). The selective inhibition of heavy acetylcholinesterase by β-endorphin thus exhibits species specificity, even among mammals, in which homologues of this molecular form of the enzyme are otherwise indistinguishable.

INTRODUCTION

AChE[4] (EC 3.1.1.7) exhibits a polymorphism in skeletal muscle tissue which has been demonstrated in several vertebrate species (1–4). It exists as three symmetrical forms, which contain 1, 2, and 4 identical catalytic subunits, and three asymmetrical forms (A_4, A_8, A_{12}), which contain 4, 8, and 12 catalytic subunits attached as tetramers to a collagen-like tail (5). In some mammalian species (6, 7), the major "heavy" form (A_{12}) is found in striated muscles only in the regions where nerve terminals are present. It appears to be localized extracellularly and is probably a component of the basal lamina (8). The heavy form of AChE may serve an important function in terminating transmission at the skeletal neuromuscular junction. Its presence is critically dependent on neurotransmission, since deprivation by pharmacological means (9) or by surgical denervation (6) causes the activity to disappear. ψChE (EC 3.1.1.8) is also

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[4] The abbreviations used are: AChE, acetylcholinesterase; ψChE, pseudocholinesterase; β-EP-, β-endorphin; β_h-EP, human β-endorphin; β_c-EP, camel β-endorphin; β_e-EP, equine β-endorphin; β_t-EP, turkey β-endorphin; β_{os}-EP, ostrich β-endorphin; β_b-EP, bovine β-endorphin; β_p-EP, porcine β-endorphin; des-ac-β_{sa}-EP-, des-*N*-acetyl salmon β-endorphin; β_o-LPH, ovine β-lipotropin; β_h-LPH, human β-lipotropin; HPLC, high-pressure liquid chromatography.

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present in mammalian (10) skeletal muscle tissues and exists as low-salt soluble and insoluble forms with sedimentation coefficients similar to those of corresponding AChE forms.

In previous work it was shown that the activity of the heavy AChE oligomer in rat skeletal muscle is reversibily inhibited by β-EP (11). The inhibition of the enzyme obeyed allosteric kinetics and could be abolished by digesting the tail subunits of the enzyme with collagenase at 37°.

In the persent investigation we set out to determine whether the inhibition of β-EP of the activity of AChE molecular forms homologous to the 16 S form of the rat could be demonstrated in human muscle as well as that of other vertebrate species. Parallel studies on ψChE were also conducted to define further the specificity of β-EP in regulating cholinesterase activity. The potency of β-EP from various species as inhibitors was investigated in order to study the species specificity of its action.

MATERIALS AND METHODS

β_h-EP, β_c-EP, β_e-EP, β_t-EP, β_{os}-EP, des-ac-β_{os}-EP, and β_h-EP analogues were synthesized by the solid-phase method (procedures based on that described for β_h-EP, ref. 12). β_h-LPH and β_o-LPH were isolated from human and sheep pituitaries, respectively, as previously described (13, 14). Bovine serum albumin, acethylthiocholine iodide, *S*-butyrylthiocholine chloride, 5,5′-dithiobis(4-alkyldimethylammonium-phenyl)pentan-3-one-dibromide, tetraisopropylpyrophosphoramide, *p*-nitrophenyl-galactopyranoside, β-NAD, catalase, β-galactosidase, equine liver alcohol dehydrogenase, benzamidine, bacitracin, pepstatin A, aprotinin, Tris base, EDTA, Triton X-100, and collagenase type VII were obtained from Sigma Chemical Company (St. Louis, Mo.). Sodium chloride, magnesium chloride, sucrose, and phosphate buffer salts were obtained from Mallinkrodt. Specimens of gastrocnemius muscle were obtained from the morgue, Moffitt Hospital, San Francisco, from one male and two female subjects aged between 65 and 80. Death was caused by stroke resulting from natural causes or through postoperative complications and took place 30 hr or less prior to dissection and freezing of the tissue specimens. Frozen eel (*Electrophorus electricans*) electric organ tissue was a gift from Dr. Z. W. Hall.

Preparation of tissue extracts and separation of low-salt insoluble proteins. Gastrocnemius muscles were rapidly dissected from female Sprague-Dawley rats aged 20 days, Silver King pigeons aged 30 days, and adult frogs (*Xenopus laevis*) after the animals were killed by decapitation. The tissues were weighed after as much fat and connective tissue as possible had been removed from mammalian and avian muscles. They were subsequently minced and homogenized at speed 8 for 90 sec on ice in a medium containing 1 M NaCl, 1% Triton X-100, 5 mM EDTA, 10 mM Tris, bacitracin (1 mg/ml), 1 mM benzamidine, pepstatin A (20 μg/ml), and aprotinin (20 μg/ml) at pH 7.5. Tissues were extracted in 1:5 medium (w/v) at 0° for 2 hr. Adult human muscles were extracted in a larger volume of medium at 4° for 5–6 hr with stirring. After extraction, residues were removed by centrifugation at 27,000 × *g* for 30 min at 4°. For preparation of low-salt insoluble proteins, the extracts were dialyzed against 5 mM Tris buffer (pH 7.5) at 4° overnight to cause precipitation of insoluble material. This was separated by centrifugation at 27,000 × *g* for 30 min at 4° and washed three times with 10 mM Tris buffer (pH 7.5) containing bacitracin. The pellet was finally dissolved in high-saline extraction medium and concentrated over a Diaflow membrane, using compressed nitrogen at 30 psi. Protein content of the muscle extracts was determined as described by Lowry *et al.*

Separation and estimation of cholinesterase molecular forms. Separation and estimation of cholinesterase molecular forms was carried out using the colorimetric method (11, 16). Aliquots (50 or 100 μl) of whole extracts or of resolubilized low-salt insoluble proteins was transferred to 5–20% linear sucrose gradients prepared in the extraction medium. The gradients were centrifuged at 2° for 16 hr at 36,000 rpm (128,000 × g_{av}) in an SW 60 rotor (tube length 60 mm, volume 4 ml) in a Beckman L5-65B ultracentrifuge. Fractions were assayed for cholinesterase activities at 23° using 0.25 mM substrate and 0.2 mM 5,5′-dithiobis-(2-nitrobenzoic acid) with a fraction dilution of 1:50. Prior to the addition of substrate, the assay mixture was incubated in the absence or the presence of peptides for 45 min at room temperature. Assay tubes contained 30 μM tetraisopropylpyrophosphoramide for inhibition of ψChE when AChE was assayed. The substrate used was acetylthiocholine and the reaction time was 45 min. Assay tubes contained 10 μM 1,5-bis(4-allyldimethylammoniumphenyl)pentan-3-dibromide for inhibition of AChE, when ψChE was assayed. The substrate was butyrylthiocholine chloride, and the reaction time was 270 min. Using the selective inhibitor (17) in combination with butyrylthiocholine as substrate, the contribution of AChE catalysis to product liberation can be expected to be negligible. Fractions were preincubated with the appropriate inhibitor together with the peptide prior to commencing the reaction. The pH of the reaction mixture was 7.5. Peptide solutions were prepared by dissolving the crystalline solid in distilled water immediately before use. β_h-LPH and β_o-LPH were incubated with the enzyme in the presence of bacitracin (500 μg/ml) and a pepstatin A (5 μg/ml).

Calibration of gradients. Sucrose density gradients were calibrated with β-galactosidase (16.14 S), catalase (11.3 S), and equine liver alcohol dehydrogenase (4.8 S). The marker enzymes were assayed spectrophotometrically as follows: β-galactosidase at 410 nm by incubation at pH 7.5 with 10 mM *p*-nitrophenylgalactopyranoside in the presence of 1.5 mM $MgCl_2$, catalase at 240 nm by incubation at pH 7.5 with 0.5 M hydrogen peroxide, and alcohol dehydrogenase at 340 mM by incubation at pH 8.6 with 1 M ethanol in the presence of 1.5 mM β-NAD.

HPLC analysis of peptides before and after incubation with enzyme preparations. Groups of four fractions from four different zones of the sucrose gradient after sedimentation of muscle extracts were pooled and dialysed overnight against 10 mM Tris-HCl buffer (pH 7.5). The dialysates were lyophilized and reconstituted in 50 μl of the same buffer. β_h-EP was added to a concentration equivalent to that used in the enzyme assay. The mixture was incubated for 2–5 hr at room temperature before being subjected to HPLC analysis together with a control incubation containing no sedimented proteins. Aliquots of 50 μl were applied to a C8 Zorbax (DuPont) column (0.46 × 25 cm) and eluted with a flow ratio of 1.5 ml/hr in 0.1% trifluoroacetic acid with a linear gradient of 25 min from 0 to 60% acetonitrile. Peptides were quantified against a β_h-EP standard by estimation of optical density at 220 nm.

Experiments were repeated with β_h-EP-(1–9) and β_h-EP-(1–27) using those fractions containing 16 S AChE.

RESULTS

Stability of peptides in the enzyme assay mixture. After a 5-hr incubation of β_h-EP with proteins sedimenting in the ranges 3, 4, 10, and 16 S on sucrose gradients, a maximal degree of degradation of 8% of the initial samples was measured. A similar amount of degradation was obtained for β_h-EP-(1–27) when incubated with fractions containing 16 S proteins. However, β_h-EP-(1–9) was subject to almost complete degradation, mainly to much smaller peptides, with an approximate rate of loss of β_h-EP-(1–9) of 20%/hr. Since enzyme assays proceeded for over 1 hr after the addition of peptides to the mixture, the relative potencies of smaller peptides as AChE inhibitors can be expected to be diminished by their relative instability.

Effect of β_h-EP and β_c-EP on the activity of AChE

molecular forms from human and rat gastrocnemius muscle extracts. Changes in the activity of the different molecular forms of AChE were investigated in human and rat gastrocnemius muscle in the presence of β_h-EP and β_c-EP at a concentration of 500 mM. In human adult gastrocnemius muscle, the most predominant AChE forms were those that sedimented at 4 and 5.5 S [G1 and G2 forms (18); see Fig. 1a]. Insoluble forms had low activity in all three cases investigated, although in other muscles obtained at postmortem, higher activities of these forms were measured (data not shown). In both human and rat, inhibition of AChE activity by β-EP was limited to the heavy form of the enzyme, which sedimented at 16 S. In human muscle, β_h-EP was more effective than β_c-EP (Fig. 1a), whereas in rat muscle, β_c-EP was more effective than β_h-EP.

Effect of β_h-EP and β_c-EP on the activity of AChE molecular forms in other vertebrates. Figure 2 shows the position of sedimentation of AChE molecular forms in tissue extracts from frog and pigeon muscle and eel electric organ. The heavy oligomer in all three cases had a higher sedimentation coefficient than that of the mammalian homologues (see Fig. 1). Nonetheless, the selective inhibition of heavy AChE by β_c-EP was seen in both frog and pigeon muscle. The experiments with eel heavy AChE were carried out at a variety of substrate concentrations and with initial enzyme specific activities varying several hundredfold. In all conditions, both β_c-EP and β_h-EP were ineffective as inhibitors.

Table 1 shows the inhibition of heavy AChE homologues of five different vertebrate species produced by β_h-EP and β_c-EP at a concentration of 500 mM. Although β_h-EP was a good inhibitor of 16 S AChE activity from human muscle, it was less effective in rat, frog, and pigeon heavy AChE assays, and was ineffective with heavy AChE extracted from eel electric organ. β_c-EP was most effective with rat 16 S AChE, but it, too, was less effective in the frog and pigeon and inactive in the eel.

Selective inhibition of the activity of the mammalian AChE heavy form. By separation and concentration of low-salt insoluble muscle proteins prior to velocity sedimentation, we were able to measure the activity of forms of insoluble AChE and ψChE in both human and rat which were not clearly demonstrable in whole muscle extracts (Fig. 3). ψChE represented a total of 6.5% and

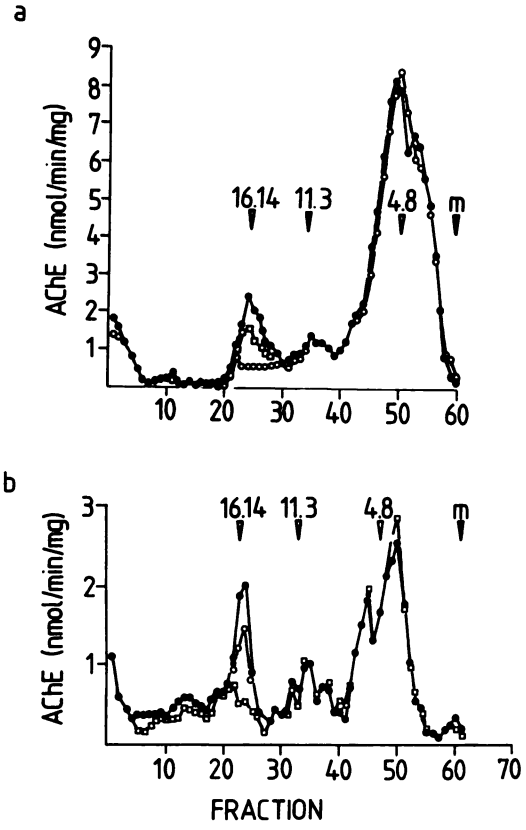

FIG. 1. *Effect of β-EP on AChE molecular forms in human and rat gastrocnemius muscle*

a. Molecular forms of human muscle AChE with different sedimentation coefficients separated by density gradient centrifugation, and assayed in the absence (●) or the presence of β_h-EP (○) or β_c-EP (□) at a peptide concentration of 500 mM. The base of the gradient is at the *left*. *Arrows* show the position of the meniscus (*m*), and of marker enzymes, identified by their sedimentation coefficients, shown in S units.

b. Molecular forms of rat muscle AChE separated as in a and assayed in the absence (●) or the presence of β_c-EP (□) or β_h-EP (○). Peptide concentrations and labeling as in a.

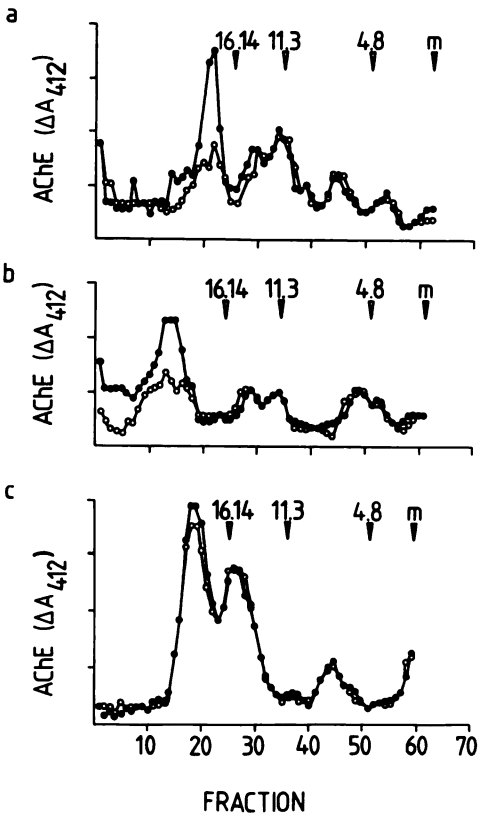

FIG. 2. *Molecular forms of AChE from nonmammalian vertebrates and the effects of β_c-EP upon their activity*

Molecular forms of AChE with different sedimentation coefficients separated as in Fig. 1. a. Frog gastrocnemius muscle. b. Pigeon gastrocnemius muscle. c. Electric eel electric organ and assayed in the absence (●) or the presence (○) of β_c-EP. Peptide concentration and labeling as in Fig. 1. The sedimentation coefficients of heavy AChE forms were 17.6 in the frog, 21 in the pigeon, and 18.7 in the eel. Inhibition of β_c-EP was demonstrable in heavy AChE homologues in the frog and pigeon but not in the eel.

TABLE 1

Inhibition of heavy AChE homologues in vertebrate species in the presence of excess β_h-EP and β_c-EP

Tissue source	% Maximal inhibition[a]	
	β_h-EP	β_c-EP
Gastrocnemius muscle		
Adult human	83	47
Rat	34	73
Frog	36	54
Pigeon	26	31
Eel electric organ	0	0

[a] Defined as the percentage of activity of heavy AChE abolished by 500 nM inhibitor. Values were obtained by integrating AChE activity from three or four fractions on either side of that containing peak heavy AChE activity.

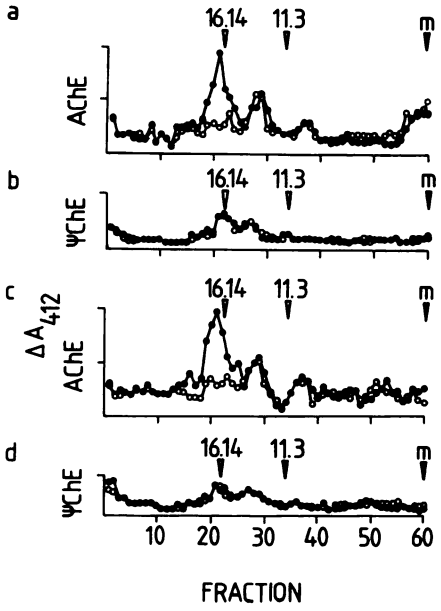

FIG. 3. *Effect of β-EP on insoluble forms of cholinesterase in human and rat gastrocnemius muscles*

a. AChE molecular forms with low-salt insoluble properties separated from human muscle extracts by dialysis, and further separated by density gradient centrifugation. Relative activities of fractions are shown in the absence (●) or presence (○) of β-EP (500 mM), demonstrating the selective inhibition of the heavy form of AChE (appearing near 16 S) by the peptide. Labeling as in Fig. 1.

b. Low-salt insoluble forms of ψChE, separated as for AChE in a, showing similar activities for all forms in the absence (●) and presence of (○) of β_h-EP. Peptide concentration and labeling as in a.

c. AChE molecular forms with low-salt insoluble properties, separated from rat muscle extracts as in a, and assayed in the absence (●) and presence (○) of β_c-EP. Inhibition of the heavy form alone is seen as in human muscle (a). Peptide concentration and labeling as in a.

d. Low-salt insoluble forms of ψChE separated from rat muscle as for AChE, showing similar activities for all forms in the absence (●) and presence (○) of β_c-EP. Peptide concentrations and labeling as in a.

4.3% of the low-salt insoluble cholinesterase total specific activity in human and rat muscle, respectively. In the absence of soluble cholinesterase components, we observed no reduction in the activity of the minor insoluble AChE forms of human muscle extracts in the presence of β_h-EP (Fig. 3a). Furthermore, there was no measureable change in the activity of any of the ψChE insoluble forms in the presence of the peptide (Fig. 3b). Thus the inhibition of cholinesterase activity was entirely specific to the heavy (16 S) AChE oligomer. Identical results were obtained when insoluble cholinesterases from rat muscle were assayed in the presence and absence of β_c-EP (Fig. 3c and d). Soluble forms of ψChE were also assayed in the presence and absence of β_c-EP, but no change in their activity was measured (data not shown).

Potency of β_h-EP and shortened analogues as inhibitors of the activity of human heavy AChE. β_h-EP inhibited the activity of AChE sedimenting at 16 S (initial activity 5 nmoles/min/mg of protein) with an IC_{50} of 0.3 mM. A considerable loss of potency was observed in a β_h-EP analogue which lacked the COOH-terminal tetrapeptide. As shown in Fig. 4, β_h-EP-(1–27) was less than 1% as potent as β_h-EP. Peptides further shortened at the COOH-terminus [β_h-EP-(1–21), β_h-EP-(1–9)] had corresponding diminished activity as enzyme inhibitors. An unusual feature of the action of these peptides was their inability to cause the same degree of inhibition as β_h-EP, even at high concentrations. This may reflect a difference in the sites at which β_h-EP and fragments thereof interact with the enzyme to cause inhibition. Peptides that lacked sequences of amino acids in the mid- and NH_2-terminal portions [β_h-EP-(1–5) to (17–31), β_h-EP-(6–31)] of β_h-EP were without inhibitory activity. β_h-LPH, when incubated with the reaction mixture in the presence of antiproteolytic agents, bacitracin and pepstatin A, also had no inhibitory activity.

Species specificity β-endorphin as an inhibitor of heavy

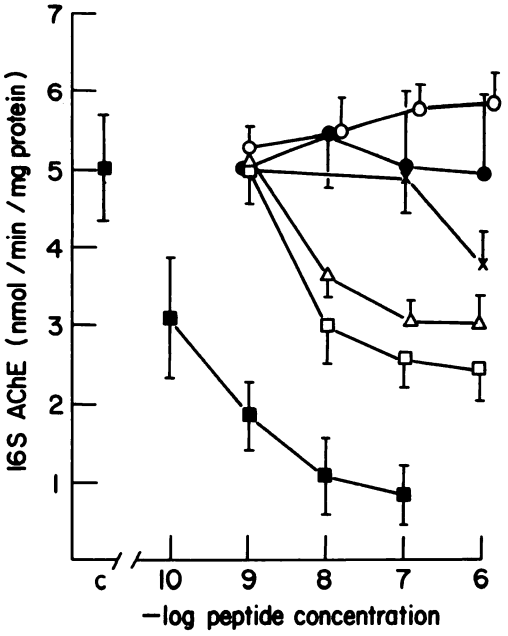

FIG. 4. *Effect of β_h-EP and analogues shortened in mid- and COOH-terminal regions in different concentrations upon human 16 S AChE*

Concentration of peptides is plotted as a logarithmic function against enzyme activity. Symbols are as follows: ■, β_h-EP; □ β_h-EP-(1–27); △, β_h-EP-(1–21); ×, β_h-EP-(1–9); ●, β_h-EP-(1–15) to (17–31) (○), β_h-EP-(6–31). Values shown are means ± standard error of the mean; n = 3–7.

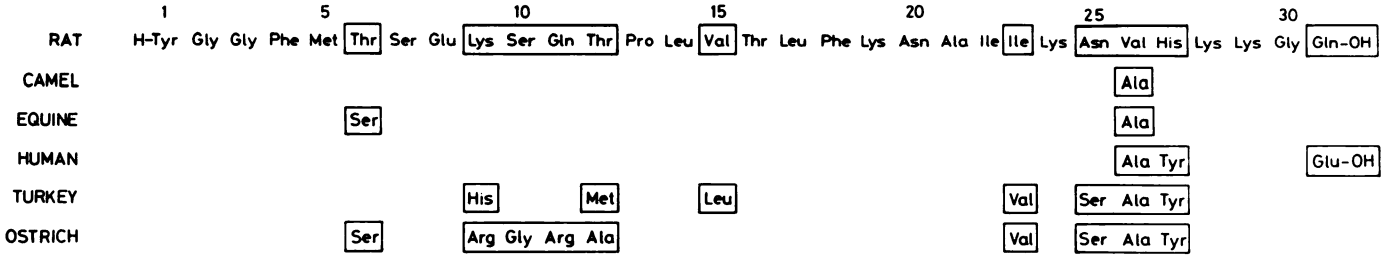

FIG. 5. *Amino acid sequence of rat pituitary β-endorphin and positions of amino acid substitution in β-endorphins of five different species used in this study*

All residues of non-rat hormones are as in the rat except those shown in *boxes*. Adapted from ref. 19 and Fig. 1.

AChE. β-EP sequences from a variety of species differed widely in their potencies as inhibitors of heavy AChE from rat skeletal muscle tissue. The amino acid sequences of the various peptides tested are compared with that of rat β-EP in Fig. 5. IC_{50} values for the peptides are given in Table 2. β_c-EP and β_e-EP were the most potent inhibitors. The IC_{50} values for these peptides resemble those previously measured for natural β_b-EP and β_p-EP (11). Minimal differences exist in the structure of the four peptides. The potency of β_h-EP was considerably less, reflecting the low inhibitory capacity of this peptide when incubated at a high (500 mM) concentration with rat 16 S AChE (see Fig. 1b). β_{os}-EP had no inhibitory activity whatsoever, as did β_o-LPH.

DISCUSSION

There is good evidence to indicate that the putative neurotransmitter/neuromodulator-like actions of β-EP are mediated through its interactions with high-affinity binding sites in neuronal membranes (20, 21). Recent studies have provided evidence that β-EP may act peripherally at sites other than cell membrane receptors. β_h-EP has been shown to bind in a saturable manner to plasma-borne molecular components of the immune system (22), and the inhibition of β-EP of a cytoplasmic enzyme, phenylalanine tRNA synthetase, has been demonstrated in a cell-free system (23). Moreover, we have reported that β-EP inhibits the activity of the 16 S or "synaptic" form of AChE in rat skeletal muscle (11).

We have presented data in this study to show that β-EP is stable throughout the experimental incubations in all fractions of the density gradient used to separate AChE. The selected inhibition of heavy AChE thus appears not to be an artifact of the degradation of β-EP catalyzed by enzymes resistant to antiproleolytic drugs in fractions from the upper parts of the gradient. However, there is no indication from these studies, on a relatively crude preparation of this enzyme, that the peptide interacts at a molecular level directly with A_{12} AChE to cause the inhibition. However, we have obtained preliminary evidence[5] to show that radiolabeled β-EP binds specifically with 16 S AChE extracted from infant human diaphragm tissue following maleylation of the enzyme and solubilization in saline-free buffer, and that the association is not abolished by digestion of part of the collagen-like component of the enzyme. A selective and direct inhibition of heavy AChE is also implied by the lack of any inhibition in the heavy form of ψChE in rat and human, an enzyme that migrates close to the sedimentation position of heavy AChE in these species and that is believed to have many properties in common with it (10).

Although their sedimentation properties and composition vary (2), a structural homology for heavy AChE in the peripheral tissues of birds and mammals and the electric organ of the teleost, *Electrophorus* (5), has been established. This homology may reasonably be expected to extend also to the heavy AChE amphibians (4). We have demonstrated the inhibition of heavy AChE, in all of these species except *Electrophorus*, by both β_h-EP and β_c-EP. As in the rat, β_c-EP was a more effective inhibitor than β_h-EP in amphibian and avian muscle, although the effectiveness of both peptides was decreased in comparison to their effectiveness in the rat. Surprisingly, there was a marked difference in the activities of these two peptides as inhibitors of rat and human 16 S AChE, β_c-EP becoming much less potent in inhibiting the human enzyme. These findings therefore suggest that there is a degree of species specificity for the interaction of β-EP with heavy AChE, part of which can be explained on the basis of measurable differences in the chemical properties of the enzyme in different vertebrate species and part of which cannot be accounted for on this basis alone. Species specificity may account for our inability to demonstate inhibition in heavy AChE from eel electric organ, although alternative explanations cannot be ruled out.

TABLE 2

Inhibitory concentrations of β-EP natural analogues and of β_o-LPH in rat gastrocnemius muscle 16 S AChE assay

The analogues were separated by velocity sedimentation and pooled from fractions containing the 16 S marker. Values are means ± standard error of the mean of concentrations that caused 50% inhibition of AChE activity. Data were collected from four to nine estimations of each value. Values are not shown where the peptide produced no measurable inhibition.

Peptide	IC_{50}
	nM
β_c-EP	3.4 ± 1.9
β_e-EP	3.9 ± 1.8
β_t-EP	6.6 ± 1.4
β_h-EP	100
β_{os}-EP	—
β_o-LPH[a]	—

[a] Incubated in the presence of antiproteolytic agents. See text.

[5] L. W. Haynes, M. E. Smith, and C. H. Li, unpublished observations.

Studies with synthetic β_h-EP fragments showed that the NH_2-terminal pentapeptide sequence was an essential requirement for inhibition of human heavy AChE. Analogues containing this sequence had inhibitory activity, but shortening of the COOH-terminus reduced inhibitory potency, partly owing to decreased resistance to muscle peptidases, to a degree depending upon the length of the peptides. Deletion of the mid-portion of β-EP also resulted in loss of inhibitory activity.

Previously we reported the inhibition of 16 S AChE in the presence of β_o-LPH in rat muscle extracts (11). Potent anticholinesterase-like effects were also seen in *in vitro* denervated skeletal muscle preparations following the addition of this peptide (24). The present data, however, reveal that β_h-LPH is ineffective as an AChE inhibitor in the presence of antiproteolytic agents. The structure-activity relationships for β-EP in this system thus resemble those observed for the binding of the peptide to rat brain membranes (20) and for production of analgesia *in vivo* (25, 26).

The phenomenon of species specificity was more strikingly demonstrated by comparing the potency of natural β-EP analogues in the inhibition of rat muscle heavy AChE. Figure 5 illustrates the positions of sequence alterations in peptides used in this study as compared with rat β-EP. In this system, mammalian β-EPs were equipotent (cf. data of ref. 11); β_t-EP, which differs in its amino acid sequence from rat β-EP at 7 positions between positions 9 and 27, was less potent. β_h-EP, which differs at positions 27 and 31, was relatively ineffective, whereas β_{os}-EP gave no inhibition at concentrations up to 1 μM. The major differences in the sequences of this natural analogue and rat β-EP lie between positions 6 and 12. This series of amino acids may contain the address sequence for the binding of β-EP to the mammalian opiate receptor (27). Thus, changes in its composition could have dramatic effects on the biological activity of the peptide. Although we have no established criteria for inhibitory potency of β-EP in the heavy AChE homologues of nonmammalian species, it is likely that β_h-EP and β_c-EP show diminished effectiveness as inhibitors in the frog and pigeon because the structural requirements differ from those of the rat homologue.

Elsewhere, we have reported evidence to suggest that physiological regulation of the heavy form of AChE by β-EP may be important during development of rat skeletal muscle (28). The present results show that this action has considerable molecular specificity and suggest a regulatory function of β-Ep in muscle tissue of other vertebrates.

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