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BETA-LIPOTROPIN POTENTLY INHIBITS A PURIFIED ANGIOTENSIN CONVERTING ENZYME FROM HUMAN BRAIN.

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The angiotensin converting enzyme (EC 3.4.15.1) is a dipeptidyl peptidase which converts the inactive decapeptide angiotensin-I into the active octapeptide angiotensin-II by removal of the COOH-terminal dipeptide His-Leu (1). The same enzyme is known to inactivate the undecapeptide bradykinin by removal of Phe-Arg and Ser-Pro (1). In addition, the converting enzyme is capable of acting on the B-chain of insulin (1), and it can act on the tetradecapeptide precursor of angiotensin to form the octapeptide angiotensin-II directly (1). The converting enzyme is present in mammalian brain (2-5) but its functional role in the CNS remains unclear. In animal and human brain highest concentrations of angiotensin converting enzyme activity are found in the caudate nucleus, putamen, nucleus accumbens, globus pallidus and substantia nigra (2-4). In addition, high levels of converting enzyme activity occur in the circumventricular organs, and in the pituitary and pineal glands (3,5).

β-Lipotropin (β-LPH) is a 91 amino acid pituitary peptide (6,7) which is also known to exist in the brain with highest concentrations in hypothalamus (8) and smaller amounts in various extrahypothalamic areas (8). B-LPH contains the active opioid peptide sequences of β-endorphin (amino acid sequence 61-91) and met-enkephalin (sequence 61-65) (6, 7). Immunocytochemical studies indicate that B-LPH is found in the medial basal hypothalamus, periventricular nucleus of the thalamus, in the area of the ansa lenticularis, zona compacta of the substantia nigra, medial amygdaloid nucleus, zona incerta, periaqueductal grey area and locus coeruleus (8).

We report here that β-LPH is a potent competitive inhibitor of angiotensin converting enzyme purified from human brain.

Enzyme purification.- The angiotensin converting enzyme was purified from a particulate fraction of human diencephalic areas which included the caudate nucleus, putamen, globus pallidus, thalamus, hypothalamus and substantia nigra. Approximately 200 g (wet weight) of brain tissue, pooled from three brains of sudden death subjects obtained within 18 h post mortem, was used as starting material. The method described by Soffer et al. (9) for the purifica-

tion of angiotensin converting enzyme from a particulate fraction from rabbit lung was used. Briefly, a 100,000 x g (90 min) pellet was obtained from a homogenate of brain tissue after discarding a 700 x g (10 min) pellet. The high speed pellet was dispersed in 10 mM phosphate buffer pH 6.5, and dialyzed for 16 h against the same buffer. Angiotensin converting enzyme was solubilized by treatment with the non-ionic detergent Nonidet-P40 (0.2% V/V), stirred for three hours and centrifuged at 100,000 x g for 90 min. The supernatant fraction was then subjected to ion exchange chromatography (DEAE-cellulose and hydroxylapatite) and gel filtration on Sephadex G-200, as described by Soffer et al. (9). The peak of converting enzyme activity in the filtrate of the Sephadex G-200 column had a V/V_o of approximately 1.387, similar to that found by Soffer et al. (9) for the converting enzyme purified from rabbit lung. The partially purified enzyme preparation had a specific activity of 1200 nmol His-Leu/min/mg, representing a 1500-fold enrichment from the starting material.

Enzyme assay.- The activity of angiotensin converting enzyme was measured as described by Yang & Neff (2) using Hip-His-Leu as the substrate.

Inhibition studies.- A total incubation volume of 0.1 ml was used routinely. The amount of enzyme present was equivalent to 0.35 µg of protein and was added last to initiate the reaction in the presence or absence of varying concentrations of β-LPH (purified from sheep pituitary, Dr C.H. Li, Hormone Research Laboratory, University of California, San Francisco, USA) and varying concentrations of substrate.

RESULTS

The results (Figure 1) suggest that β-LPH is a competitive inhibitor of the hydrolysis of the substrate Hip-His-Leu by the angiotensin converting enzyme purified from human brain. The calculated K_i value for β-LPH is 0.78 µM. β-endorphin, met-enkephalin and the analogue D-Ala^2-met-enkephalin did not significantly inhibit the enzyme when tested at concentrations up to 40µM (data not shown).

The finding that β-LPH, a substance which is unevenly distributed in the brain, is capable of inhibiting the angiotensin converting enzyme from human brain suggests that β-LPH may be a substrate for the enzyme. This possibility is of interest since we have previously found that converting enzyme activity in human brain is reduced in the corpus striatum (3,4) and substantia nigra (4) of patients dying with Huntington's disease and in the substantia nigra reticulata of patients with schizophrenia, particularly in those with an early onset of the illness (10). The fact that met-enkephalin and the analogue D-Ala^2-met-enkephalin do not inhibit the enzyme purified from brain argues against the suggestion that the converting en-

zyme is involved in the degradation of enkephalin (11, 12). If β-LPH represents another of the naturally occurring substrates for the converting enzyme, it will be of interest to determine what the products of this reaction are. Such studies are presently being undertaken.

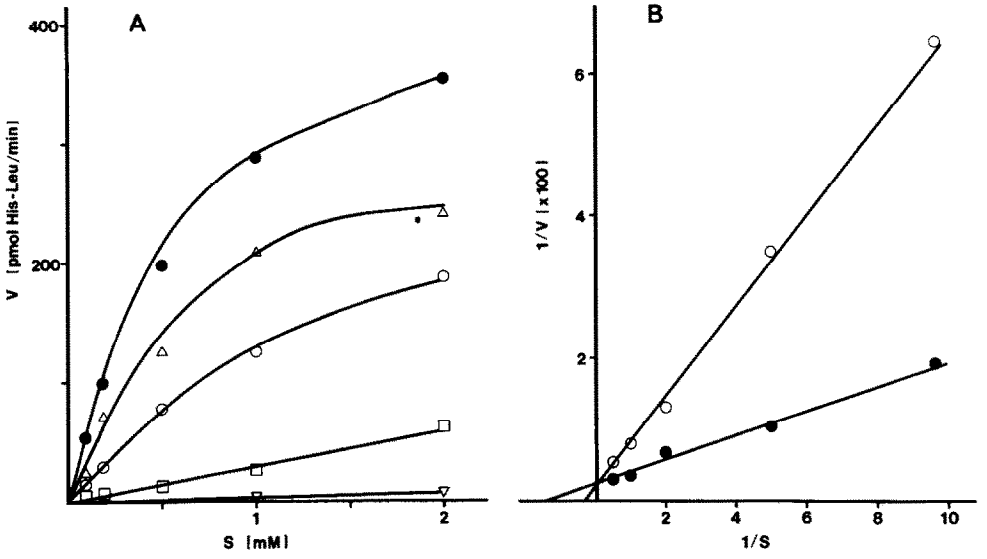

FIGURE 1. (A) Hydrolysis of Hip-His-Leu at different concentrations in the absence (●) and presence of β-LPH at the following concentrations: 40 μM (▽), 5 μM (□), 2 μM (○) and 1 μM (△).

(B) Lineweaver-Burk plot of the hydrolysis of Hip-His-Leu in the absence (●) and presence of 2 μM B-LPH (○).

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