Structural Characterization and Biological Activity of Cupric Complexes of Leu- and Met-enkephalins

P. SHARROCK, R. DAY, S. LEMAIRE, S. ST-PIERRE

Département de Chimie, Faculté des Sciences and Département de Physiologie-Pharmacologie, Faculté de Médecine, Université de Sherbrooke, Québec, Canada

H. MAZARGUIL, J. E. GAIRIN and R. HARAN

Laboratoire de Pharmacologie et Toxicologie Fondamentales du CNRS and Laboratoire de Chimie de Coordination du CNRS, Université Paul Sabatier, 205, route de Narbonne, 31400 - Toulouse, France

Received October 1, 1981

The formation of cupric ion complexes with leucine-enkephalin and methionine-enkephalin was analysed by ^1H NMR, EPR and visible spectroscopy. The biological activity of the complexes was measured by the guinea pig ileum assay and the opiate binding test to rat brain homogenates. Small changes in biological activities are reported in spite of strong binding of the copper ions to the pentapeptides.

Introduction

Since the isolation and characterization of the two endogenous, morphine-like peptides methionine-enkephalin (Met-EK) and leucine-enkephalin (Leu-EK) [1], considerable research effort has been directed at relating their biological actions—mainly analgesia—to their tridimensional structure. It was shown that both EK s interact with the same receptors as do natural opiates, such as morphine, and their action is also antagonized by naloxone [2]. Recent conformational analyses by several authors [3, 4] provided evidence that Leu- and Met-EK can adopt preferentially a folded, β-turn type structure in aqueous solution. Parallel increases of biological activity take place following the substitution of the residue in position 2 of the β-turn (Gly) by D-Ala and subsequent stabilization of the turn [5]. It is believed that in addition to stabilizing the biologically active conformation, this substitution prevents most of the enzymatic degradation of the peptides, at the Tyr-Gly level, by endogenous enkephalinases [6].

Recent work shows that metal ions influence the inhibition of naloxone binding [7] and may change the conformation of the peptides or the receptor sites by specific salt effects [8, 9]. The formation of 'inner' and 'outer' complexes of enkephalin could modulate the biological activities through formation of metal–peptide or peptide–metal–receptor complexes of different stabilities and flexibilities. Previous results show that Zn(II) ions rigidify the structure of Met-EK and (D-Ala^2, Pro^5) analogues [10] in a manner which should lead to increased μ agonist activity and decreased δ agonist potency [11]. Also, aluminium forms two different types of complexes with Leu-EK [12]. The synthesis of metal derivatives of enkephalins is a new field which could explain pharmacological variations in biological activities.

In the present work, we wanted to verify whether the complexation of Leu- or Met-EK with a transition metal cation such as Cu(II) could have a stabilizing effect on the enkephalin β-turn and eventually result into improved biological properties.

Experimental

Materials and Methods

Leu- and Met-enkephalins were both synthesized by H. Mazarguil, using classical solution techniques, as described previously [13].

Fresh D_2O solutions of the enkephalins were used for NMR in the concentration range of 0.08 to 0.1 *M*. DCl and NaOD solutions were used to adjust the pH, which was not corrected for deuterium isotope effects. ^1H NMR spectra were recorded on a Bruker 400 MHz spectrometer, with the number of scans varying between 40 and 100. Absorption spectra were recorded either with a Cary 14 (1 cm pathlength cells) or a Unicam (microcells) spectrophotometer, using variable concentrations of EK and Cu(II). An E-9 Varian EPR spectrometer was used with quartz sample holders for the paramagnetic resonance experiments.

The opiate binding assay was based on the ability of the peptides or the copper complexes to inhibit [^3H]-naloxone binding to rat brain homogenates, as previously described [14]. The IC_{50} values (concentration which produces a half-maximal inhibition) were derived from log probit plots of values ob-

0020-1693/82/0000-0000/$02.75