CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Enkephalins in Rat Brain by Fluorescence Detection

Purpose

The enkephalins are a group of opioid peptides that occur at extremely low concentrations in the brain and blood, and therefore pose a challenging analytical problem. Precolumn derivatization with naphthalenedialdehdye/cyanide* (NDA/CN, F1) followed by multidimensional LC and fluorescence detection, was evaluated for the determination of enkephalins in brain samples.

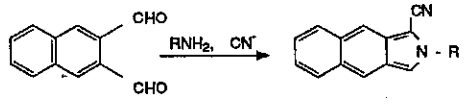

Figure 1. Reaction of NDA/CN with primary amines.

Existing methods

Radioimmunoassay is the most commonly employed method for the measurement of the enkephalins. It has adequate sensitivity but cannot distinguish between the various enkephalin peptides. Liquid chromatography has been coupled to RIA in order to achieve greater specificity. Radioreceptor assays and mass spectrometry have also been used to detect enkephalins in brain and blood samples following LC.

Conditions

Detector: BAS FL-45 Fluorescence Detector:
excitation wavelength =420 nm,
emission wavelength = 490 nm
Columns: (1) Spherisorb Phenyl (5 m, 150 X 4.6 mm)
(2) ODS Hypersil (5 m, 150 X 4.6 mm)
Mobile phase: 45% (v:v) acetonitrile, 55% 26.5 mM TFA (pH 3.5). Flow rate was 1.0 mL/min

Preparation of Rat Brain Samples

A sample of the rat striatum (ca. 0.2 g) was obtained from the brain of a male Wistar rat and homogenized in 10% hydrochloric acid. Alanine-met-enkephalin ((A)-ME), 500 picomoles, was added as an internal standard. The exact procedure used is described in reference 1.

Derivatization procedure (BAS P/N CF-1044)

20 µL of sample
20 µL methionine enkephalin (ME) and/or leucine enkephalin (LE)
20 µL (A)Me (internal standard)
50 µL of 200 mM ascorbic acid
100 µL of 10 mM potassium cyanide
200 µL of 5 mM NDA
540 µL of 100 mM phosphate buffer pH 6.8

Reaction is allowed to proceed for 20 minutes at 4°C and then quenched by the addition of 50 µL of 200 mM taurine.

Analysis of Rat Brain by Multidimensional LC

F2A shows the multidimensional separation of the enkephalins in rat brain following precolumn derivatization with NDA/CN. Peak 1 is LE and peak 2 is the internal standard (A)-ME and peak 3 is ME. The levels correspond to 429 pmol ME and 130 pmol LE per gram of wet tissue. F2B is the same sample that has been spiked with 2 pmols of CBI-LE and CBI-ME.

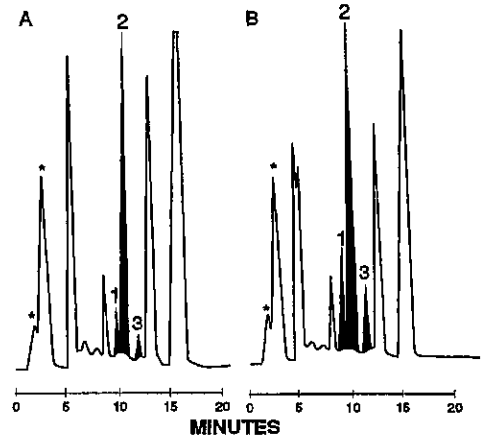

Figure 2. Chromatograms of LEU-enkephalin (1) and ME-enkephalin (3) in the striatum region of the rat brain.

Notes

Samples were stable for up to 6 hours if kept cold and protected from light.

It is critical that the derivatization is performed near pH 7, otherwise cyclization reactions will occur which reduce the yield of the derivative (2).

It is also important to have the NDA and CN in a 1:1 ratio. If higher concentrations of cyanide are employed, the reaction rate is reduced considerably. We have found that concentrations of 1 mM NDA and 1 mM cyanide are optimal.

References

1. M. Kai, J. Ishida and Y. Ohkura, J. Chromatogr. 430 (1988) 271.

2. P. De Montigny, C.M. Riley, L.A. Stemson and J.F. Stobaugh, J. Pharm. Biomed. Anal. 8 (1990) 419-429.

3. M. Mifune, D.K. Krehbiel, J.F. Stobaugh and C.M. Riley, J. Chromatogr. 496 (1989) 55-70.

4. L.M. Nicholson, H.B. Patel, F. Kristjansson, S.C. Crowley, K. Dave, J.F. Stobaugh and C.M. Riley, J. Pharm. Biomed. Anal. 8 (1990) 805-816.

* US PATENT # 4837166