Determination of the Concentrations of Dynorphins at the Low Physiological Concentrations by LC-MS/MS

Karthik Chandu[1], Tony Sahley[2], Michael Hammonds[3], Dr. Masaru Miyagi[4], David Anderson[5]

Department of Chemistry, Cleveland State University; School of Health Sciences and Department of Biological, Geological and Environmental Sciences, Cleveland State University; Department of Pharmacology, Case Western Reserve University

Abstract

Dynorphins are endogenous opioid peptides that have been implicated as initiators of immune and inflammatory response through upregulation of inflammatory cytokine and chemokine production, as well having a role in glutamate-induced neuro-inflammation and neurotoxicity. Previously published HPLC-mass spectrometry techniques have insufficient detection capabilities for quantification and detection of dynorphins at physiologic concentrations. A sensitive LC-MS/MS technique has been developed in the present work which can separate and quantify dynorphin A, dynorphin B and neoendorphin by mass spectrometry below their low physiologic concentrations, being 4000 times more sensitive than previously published HPLC-mass spectrometry techniques.

Introduction

Dynorphins are endogenous opioid peptides. Dynorphins play a role in a variety of physiological processes such as regulation of pain, temperature, motor activity, the cardiovascular system, respiration, feeding behavior, hormonal balance and responses to shock and stress.

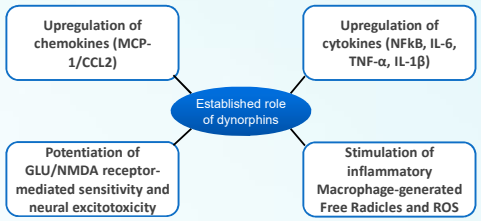

Figure 1: Processes regulated by dynorphins

Analytical Background

Dynorphins are very potent peptides and are present in very low concentrations ranging from 0.1 fmol/mL to 20 fmol/mL in human serum in absence of stimulation in disease process. (1)

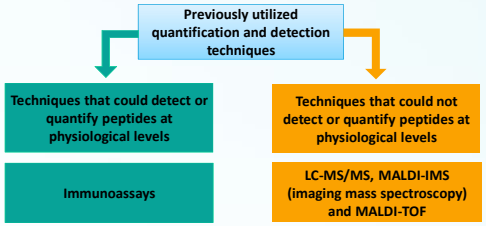

Figure 2: Previously utilized quantification and detection techniques

Previous LC-MRM/MS work reported a limit of detection of 61 fmol/ml (2) which is substantially higher than the physiological levels. The current technique able to quantify the peptides at the physiological concentrations and the limit of quantification is 4000 times lower than previously published techniques.

Experimental conditions

Instruments used:

- Sciex ESI-triple quadrupole mass spectrometer
- Shimadzu Nexera UPLC system

Column:

- Luna® Omega 1.6 micron Polar C18 100 Å, LC Column
- Dimensions: 50 x 1.0 mm

Solvent system:

- Mobile phase A: 100 % water + 0.1% formic acid
- Mobile phase B: 100 %ACN + 0.1% formic acid
- Flow rate : 0.1 ml/minute

Research work flow

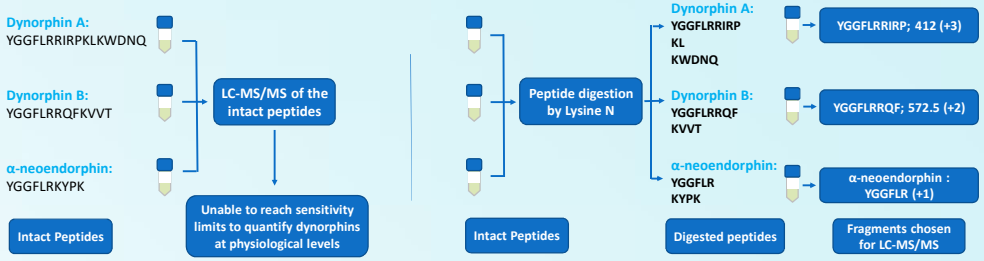

Figure 3 : Work flow for intact peptides

Figure 4 : Work flow for fragmented peptides

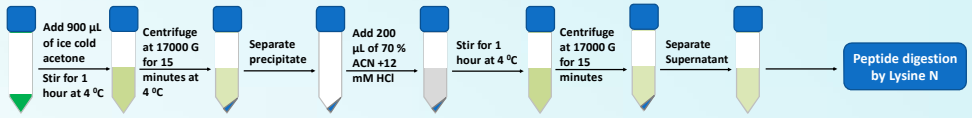

Figure 5 : Sample preparation procedure for differential solubility method

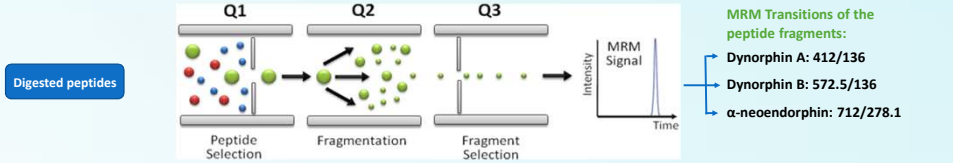

Figure 6: LC-MS/MS of the peptide fragments (figure adapted from Schmidt, A., Picotti, P., & Aebersold, R. (2008). Proteomanalyse und Systembiologie. BIOspektrum, 14(1), 44

Results

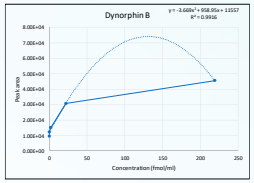

Figure 7: Standard graph for dynorphin B

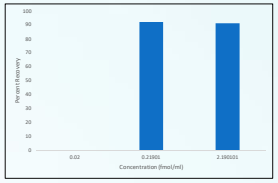

Figure 8: Percent recovery in sample preparation method for 3 concentrations of dynorphin B

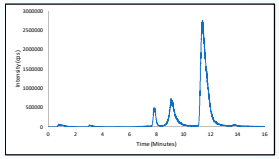

Figure 9: Separation of dynorphin A, B and α-neoendorphin

Conclusion

1. Dynorphins and α-neoendorphin have been separated
2. Ultra sensitivity for quantification and specificity for each peptide has been achieved by the proteolytic digestion of the peptides with Lysine N.
3. This is the first LC-MS/MS technique developed to able to accurately and precise quantify dynorphin B at a concentration 1.5 lower than the lowest physiological concentration.
4. Quantification close to physiological concentrations with a sample recovery of greater than 90 % from serum has been achieved.

Acknowledgements

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References

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2. Chao CC1, Gekker G, Hu S, Sheng WS, Portoghese PS, Peterson PK. Upregulation of HIV-1 expression in cocultures of chronically infected promonocytes and human brain cells by dynorphin. Biochem Pharmacol. 1995;50(5): 715-22.