

Application Note

Radioimmunoassay (RIA) of Dynorphin peptides

Introduction

Dynorphins are the body's natural opioid peptides with pain relieving activity. The study of Dynorphin levels are central to a range of diverse research fields, such as: pain, addiction, immunology and behavioral research. The dynorphin system is one example, among many peptide systems, with diverse function and tissue distribution. As transient signaling molecules peptides are subject to degradation by natural enzymatic activity in the sample. Measured levels can be severely affected by remaining enzymatic activity post-sampling. In this application, the Stabilizor™ system was evaluated on its ability to prevent changes to peptide levels. This application note is based on work performed in the lab of Prof. Ingrid Nylander, Uppsala University, Sweden.

Results

Heat stabilization preserves tissue from the moment of sampling and instantly stops enzyme activity. It is a permanent, additive-free method inhibiting molecular instabilities and post sampling changes to peptides.

In the featured study from Uppsala University, the levels of three peptides in the dynorphin system have been measured using RIA. Levels have been compared between samples collected using best practise with rapid snap freezing and samples subjected to heat stabilization directly after harvesting. There are large differences between snap frozen and heat stabilized samples in the measured levels of the three dynorphin peptides, fig 2. In stabilized samples, levels of intact dynorphin peptides, A and B, are higher than the further processed Leu-Enk-Arg peptide. In snap frozen samples, the opposite trend can be seen, with lower levels for the intact peptides and high level of the metabolite. In light of the enzymatic nature of the continuous conversion of intact dynorphin A and B into Leu-Enk-Arg, this is clear evidence of continued degradation post sampling and during extraction.

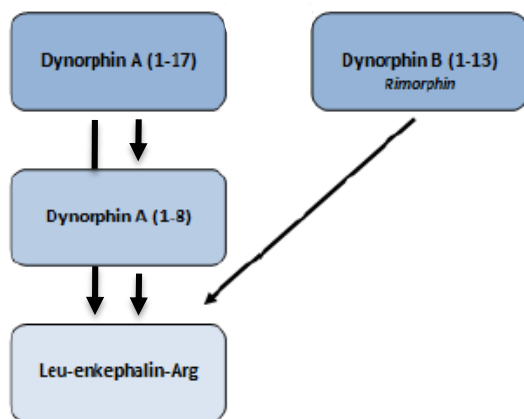
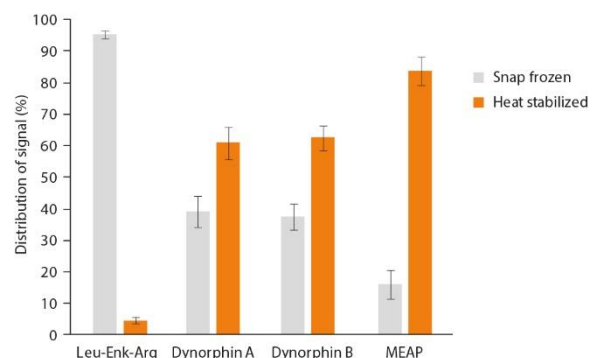


Figure 1. Dynorphin processing flow chart

Figure 2. Dynorphin measured with RIA clearly show rapid degradation in the snap frozen sample, resulting in high levels of the metabolite Leu-Enkephalin-Arg. In heat stabilized samples the levels of parent peptides, Dynorphin A, Dynorphin B and MEAP remain high.



Methods

1. Mice hypothalamus was excised and either snap frozen or heat stabilized using the Stabilizer T1 in Quick Fresh (compress) mode.
2. Samples were frozen in 1.5 mL test tubes.
3. To the frozen sample 1 M hot (95°C) HAc was added (1 mL/50 mg tissue, minimum 1 mL).
4. The sample was heated in water bath (95 °C) for 5 minutes.
5. The samples were cooled on ice
6. The samples were homogenized with a Branson Sonifier (Danbury, CT, USA).
7. The homogenate was reheated at 95°C for an additional 5 minutes
8. The samples were cooled on ice
9. The homogenate was centrifugated for 15 min at 4 °C at 12000 rpm.
10. The supernatant was collected in Minisorb tubes (Nunc, Denmark)
11. The sample was purified using ion exchange chromatography (IEC).
 - i. The extract was added to repackaged mini-columns with SP Sephadex C-25 gel.
 - ii. The sample was eluted in three steps using a mix of formic acid and pyridine with increasing ionic strength according to [Bergström et al., 1983; Christensson-Nylander et al., 1985].
 - iii. The eluate was collected in 5 mL fractions
12. Fractions were desiccated in a vacuum centrifuge (Savant SpeedVac Plus SC210A).
13. Fractions II, III, and V, containing Leu-enkephalin, MEAP and Leu-Arg, and Dyn A and Dyn B respectively, were used for further RIA analysis.
14. Prior to RIA analysis, samples were stored desiccated in the freezer (-20°C).
15. RIA analysis was conducted according to [Nylander et al., 1997]

References

Bergström, L., Christensson, I., Folkesson, R., Stenström, B., Terenius, L., 1983. An ion-exchange chromatography and radioimmunoassay procedure for measuring opioid peptides and substance P, *Life Sci*, 33, 1613-1619.

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Nylander, I., Stenfors, C., Tan-No, K., Mathé, A.A., Terenius, T., 1997. A comparison between microwave irradiation and decapitation: basal levels of dynorphin and enkephalin and the effect of chronic morphine treatment on dynorphin peptides, *Peptides*, 31:4, 357–365