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Increased success rate of finding endogenous peptides in tissue samples

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Endogenous peptides are often present at low levels *in vivo*. Normal peptidase/protease control mechanisms continue after tissue excision and post-mortem proteolytic activity begins immediately, producing protein fragments (peptides) that can mask the endogenous peptides during analysis.

Tissue preparation, extraction and isolation steps have been optimized to achieve high yields of non-degraded extracts in order to facilitate reproducible detection and measurement of endogenous peptides. The resulting protocol was developed using rapid heating to stabilize tissue samples from the moment of sampling (Stabilizor system, Denator), before homogenization, extraction and peptide isolation.

To determine the optimal conditions for peptide extraction, three different extraction/homogenization buffers were tested on tissue heat-stabilized at different time points after sampling. Homogenization intensity and duration were varied to investigate the possibility of inducing breakage of peptide bonds. Ultra-filtration filters with a 10 kDa cut-off limit from two suppliers were compared. Methods were optimized using mouse cortical tissue.

To determine the effect of heat stabilization on subsequent analysis, fresh brain tissue from Wistar rats was 1) snap frozen or 2) snap frozen and heat stabilized. After dissection, samples were homogenized and peptides extracted using a Stabilizor Peptide Extraction Kit (Denator). Peptides were analyzed using nano-LCMS and MS/MS and data analyzed using Progenesis LC-MS version 4.0 (Nonlinear Dynamics).

Several neuropeptides originating from precursors were identified in specific brain regions (hypothalamus and pituitary gland), but only in heat-stabilized samples. Further analysis revealed that other peptides from the same precursor appeared to be expressed similarly suggesting potential biological relevance.

Hundreds of unique peptides including several neuropeptides, originating from peptide- or hormone- containing precursors, have been identified in heat-stabilized samples. In standard snap-frozen samples, using the same extraction procedure, numerous protein fragments from degradation have been found.

In conclusion, heat stabilization of fresh or snap-frozen tissue significantly reduces sample complexity, minimizes the risk of false identification and avoids masking of endogenous peptides present at low levels *in vivo*.