# In vitro recovery measurement of Peptides

APPLICATION NOTE

## Measurement of peptide release by microdialysis presents several problems compared to the dialysis of low molecular weight compounds:

**1.** Peptides have larger molecular weights and therefore the membranes recover less of them (for details about principles of recovery and the effect of molecular weight see Application Note No. 1).

**2.** Low recovery and low physiological concentrations of extracellular peptides require the use of highly sensitive immunoassays.

**3.** A number of peptides are "sticky " and adhere to the membrane material and other parts of the probe. This reduces the ability of the technique to follow the dynamic concentrations of these peptides in the tissue.

## Pratical measurement of in vitro recovery:

**1.** The simplest and quickest method is to place a radio-labelled peptide in a solution external to the probe and compare the counts recovered in the perfusate with the counts present in the same volume of the external medium (e.g. using a gamma counter).

**2.** With "sticky" peptides the addition of 0.5% bovine serum albumin to the perfusion medium may help.

**3.** Typical flow rates for microdialysis sampling of peptides are between 3 and 6 µl/min.

**4.** See Appl. Note No.1, for more details.



## In vitro recoveries (%) of peptides by microdialysis probes

| | CMA/Microdialysis 20 kDa cut-off (0.5 mm o.d.) Membrane Length | | | 50 kDa cut-off (0.3 mm o.d.) M. L. | | 5 kDa cut-off (0.25 mm o.d.) M. L. | |
|---|---|---|---|---|---|---|---|
| Neuropeptide * "sticky" peptides | 5 mm | 2 mm | 1mm | 5 mm | 2 mm | 5 mm | 2 mm |
| THR (362) | 19.4 | 11.8 | 6.7 | 4.5 | 2.3 | 3.8 | 1.9 |
| [Leu]enkephalin (553) | 20.9 | 10.5 | 5.9 | 6.0 | 3.1 | 5.8 | 3.0 |
| [Met]enkephalin (574) | 24.8 | 13.0 | 7.1 | 6.5 | 3.3 | 6.4 | 3.2 |
| Oxytocin (1007) | 16.4 | 8.6 | 5.0 | 4.0 | 2.0 | 0.9 | 0.5 |
| Angiotensin II (1046) | 19.0 | 9.4 | 5.4 | 3.6 | 1.7 | 1.9 | 0.9 |
| AVP (1084) | 18.3 | 9.1 | 5.1 | 3.9 | 1.9 | 1.1 | 0.6 |
| Neurokinin A (1133) | 18.0 | 9.1 | 5.2 | 4.0 | 2.0 | 1.2 | 0.5 |
| CCK-8 (1142) | 12.7 | 6.4 | 3.7 | 1.3 | 0.8 | 0.7 | 0.3 |
| LHRH (1182) | 15.6 | 8.0 | 4.6 | 3.9 | 1.9 | 1.0 | 0.5 |
| Substance P (1348) | 15.5 | 7.5 | 4.5 | 3.4 | 1.6 | 0.8 | 0.4 |
| Bombesin (1620) | 16.6 | 8.1 | 4.5 | 3.3 | 1.5 | 1.2 | 0.6 |
| Neurotensin* (1673) | 12.0 | 6.3 | 3.5 | 2.6 | 1.3 | 0.6 | 0.3 |
| Dynorphin 1-17 (2148) | 6.5 | 3.3 | 1.9 | 1.1 | 0.6 | 0.2 | 0.1 |
| β-Endorphin* (3466) | 3.0 | 1.4 | 0.9 | 0.2 | 0.1 | <0.1 | <0.1 |
| NPY* (4271) | 1.5 | 0.7 | 0.4 | 0.1 | <0.1 | <0.1 | <0.1 |
| CRF (4758) | 3.1 | 1.6 | 0.9 | 0.2 | <0.1 | <0.1 | <0.1 |

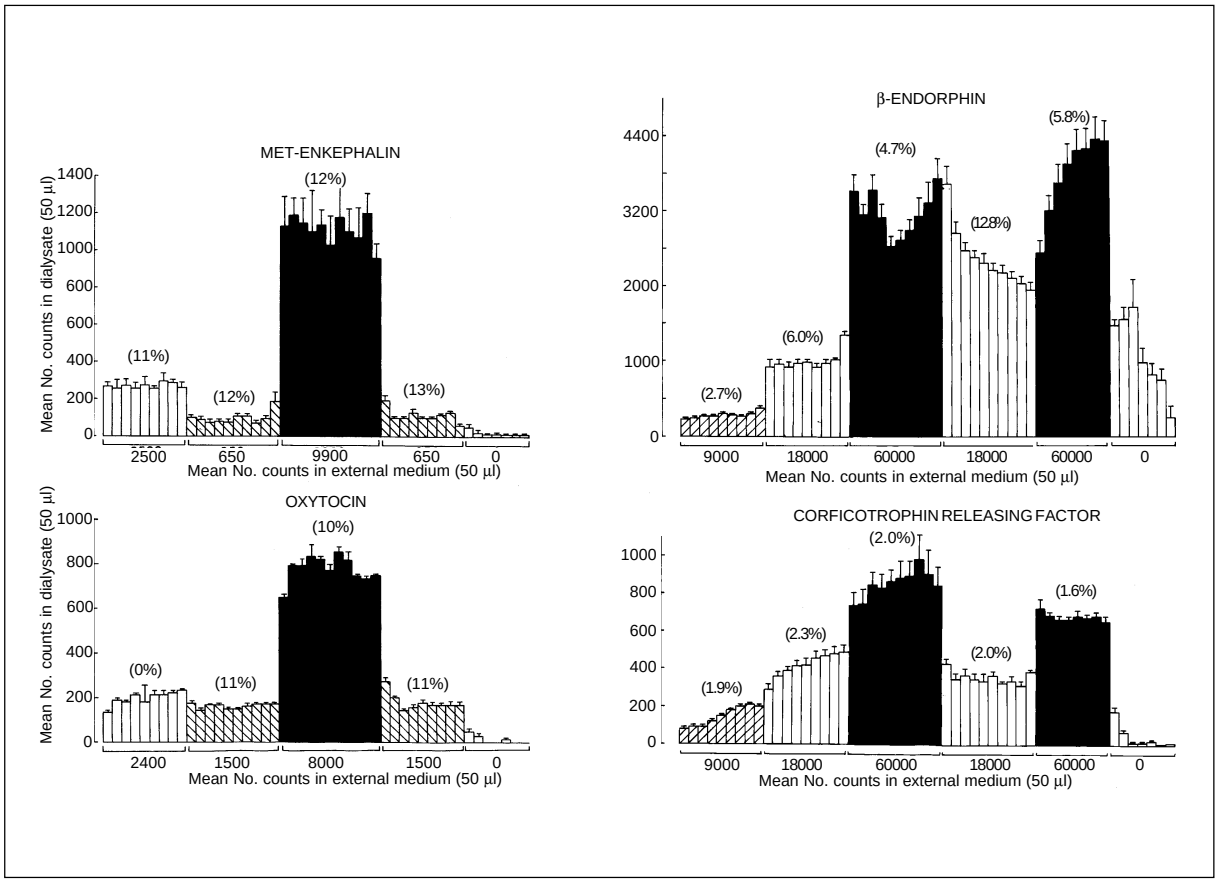


*Fig. 1. Mean ± S.E.M. in vitro recoveries of three CMA/Microdialysis Probes placed in three different concentrations of radio labelled peptides. Perfusion medium was Ringer (β-endorphin: Ringer + 0.5 % BSA) at a flow rate 4 µl/min, 15 min fractions were collected. Figures in brackets show mean % recoveries for each particular concentration.*



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(Material in this application note kindly provided by Dr K.M. Kendrick, Institite for Animal Physiology and General Research, Cambridge U.K.)

**If you require further details on Microdialysis procedures, HPLC analysis, instrumentation or bibliography, please do not hesitate to contact:**

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