

## In vitro recovery measurement of Peptides

### 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.

### Practical 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  $\mu\text{l}/\text{min}$ .
4. See Appl. Note No.1, for more details.

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

Neuropeptide * "sticky" peptides	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.	
	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
$\beta$ -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

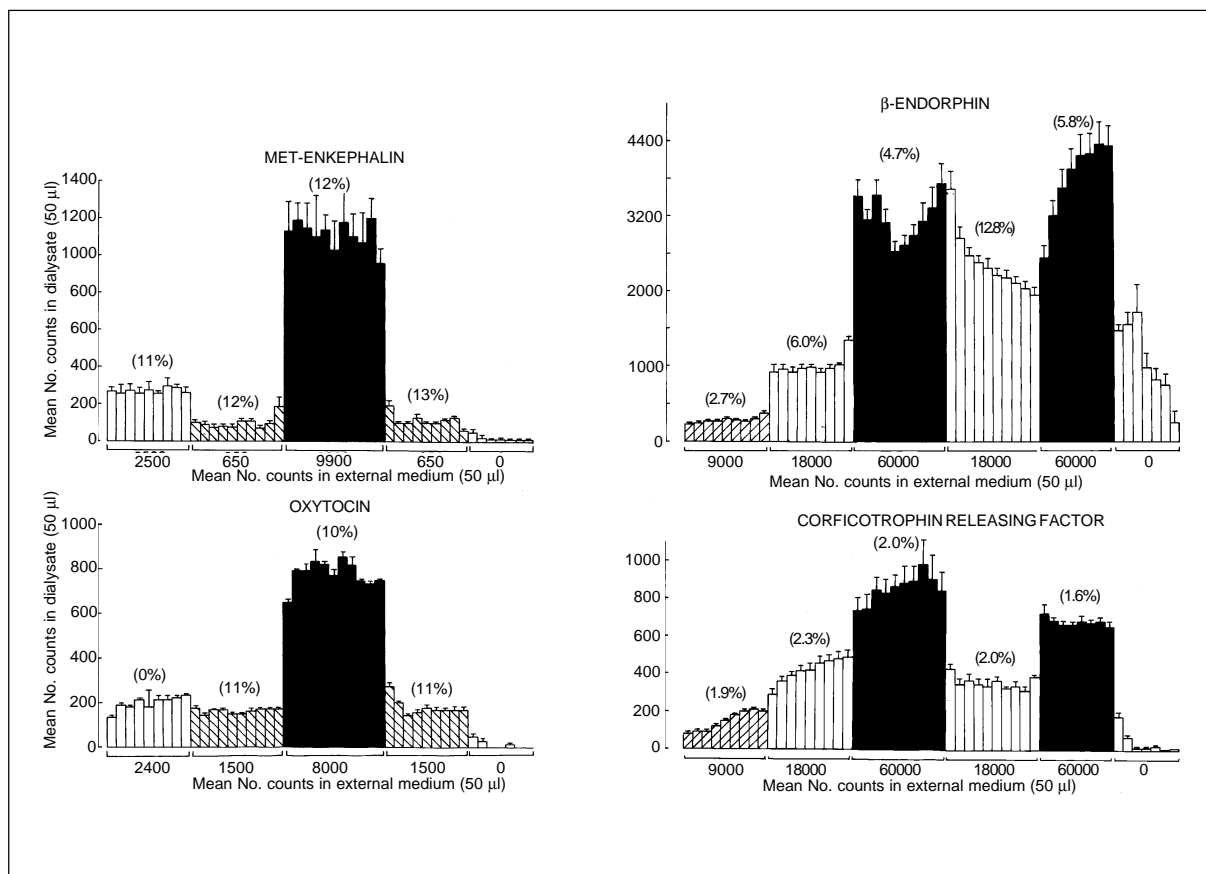


Fig. 1. Mean  $\pm$  S.E.M. *in vitro* recoveries of three CMA/Microdialysis Probes placed in three different concentrations of radio labelled peptides. Perfusion medium was Ringer ( $\beta$ -endorphin: Ringer + 0.5 % BSA) at a flow rate 4  $\mu$ l/min, 15 min fractions were collected. Figures in brackets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

(Material in this application note kindly provided by Dr K.M. Kendrick, Institute 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:**

**CMA/Microdialysis**

www.microdialysis.com

Box 2 • 171 18, Solna  
Tel 08-470 10 00 • Fax 08-470 10 50

73 Princeton Street, North Chelmsford • MA 01863 • USA  
Tel: +1 (978) 251-1940, (800) 440-4980, Fax: +1 (978) 251-1950