Assessment of cellular environments on radioligand binding – an application to opioid receptor PET imaging

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Introduction

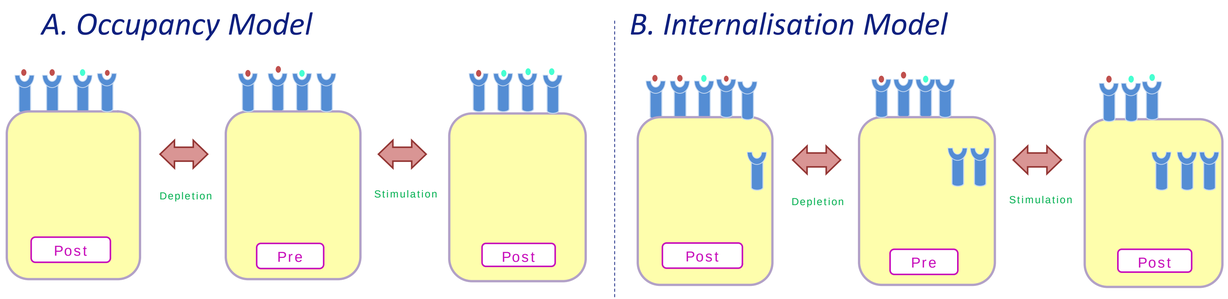
• Imaging the release of endogenously produced opioid peptides would vastly increase our understanding of the opioid system in various neurobiological disorders.

• PET radiotracers such as [^{11}C]diprenorphine and [^{11}C]carfentanil are allowing the release of endogenous opioid peptides to be investigated.

• Both [^{11}C]diprenorphine (1) and [^{11}C]carfentanil (2) have been shown to be sensitive to acute changes in peptide concentrations using physiological release paradigms.

• Any change in binding potential (BP = B_{max}/K_d) observed in endogenous release imaging studies is generally accepted to take place by a direct competition mechanism; The 'Occupancy Model' (Figure 1A). However, an alternative 'Internalisation Model' has been proposed (Figure 1B; (3)):

Figure 1. Change in BP hypothesis



The μ/δ/κ-Opioid receptors (OR) are featured in blue. PET radiotracer binding is depicted as red spots, 'endogenous neurotransmitter' binding is depicted as green spots. 'Depletion' indicates reductions in synaptic transmitter levels. 'Stimulation' indicates an increase in synaptic transmitter levels. During a pre-challenge/baseline scan, receptors at the cell surface are occupied by both PET radiotracer and transmitter. A. Occupancy Model. Following stimulation increased transmitter levels displace PET radiotracer at the cell surface. Following Depletion of transmitter, an increase in PET tracer binding is observed. B. Internalisation Model. Following stimulation, increased levels of transmitter in the synaptic cleft out-compete PET radiotracer for surface binding and cause agonist induced internalisation or ORs, reducing regional PET signals. Following depletion, decreases in synaptic transmitter from baseline levels leads to an increase in the number of unoccupied μ, δ and κ on the cell surface and more PET radiotracer is able to bind, increasing regional PET signals.

•Opioid receptors (μ-, δ- and κ-OR) are known to internalise following acute exposure to endogenous or exogenous agonists (4-7).

• Internalisation processes may play a role in the reduction in BP observed during acute release studies with PET.

• Following agonist-induced internalisation, a reduction in affinity (K_d) of receptor for radiotracer or a reduction in receptor availability (B_{max}) in intracellular environments compared to the extracellular may contribute to an overall decrease in BP.

Aims

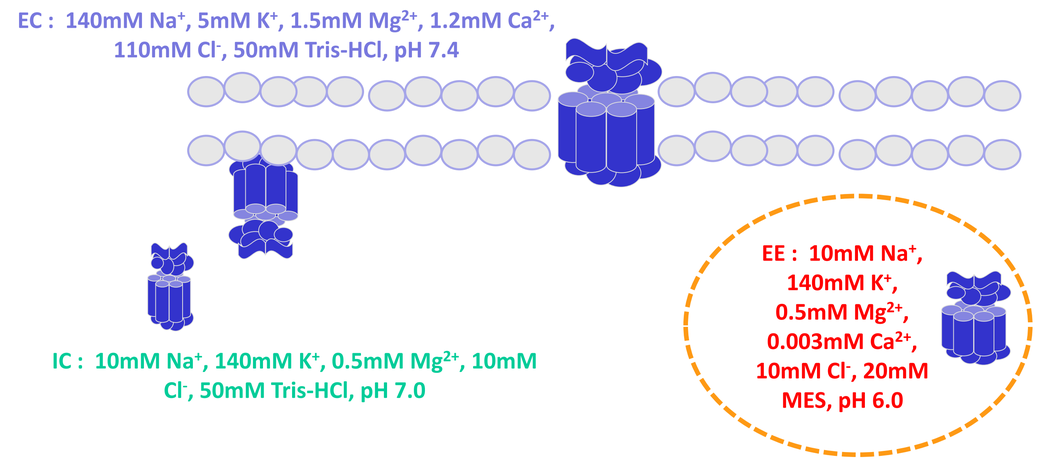
(1)To examine the effect of different cellular environments involved in the receptor internalisation pathway on the binding parameters of the two OR PET radiotracers, [^{11}C]diprenorphine ([^{3}H]diprenorphine used here) and [^{11}C]carfentanil *in vitro*.

(2)Assess the affinity (K_i) of a range of endogenous opioid peptides for the μ-OR using [^{11}C]carfentanil, *in vitro*.

Methods

Buffers representative of three different cellular environments Extracellular (EC); Intracellular (IC) and Endosomal (EE) were generated (Figure 2).

Figure 2. Ionic Compositions Representative of Different Cellular Compartments (37° C)



Receptors featured in blue as plasma membrane bound for EC, Intracellular membrane associated or cytoplasmic localisation for IC and endosomal association for EE binding conditions.

Radioligand binding assays were performed at 37°C using rat whole brain homogenate membrane preparations. Eight concentrations of [^{3}H]diprenorphine (0.001-3nM, incubation 90 minutes) and [^{11}C]carfentanil (0.003-10nM, incubation 30 minutes) were used for saturation studies. Non-specific binding was determined using naloxone (10μM). For competition studies a fixed concentration of [^{11}C]carfentanil was used (0.14 ± 0.03) and a concentration range of β-endorphin (10pM-10μM), endormorphin-1 and -2 (3pM-100μM) and met- and leu-enkephalin (3pM-100μM).

Results

• Receptor availability (B_{max})for both [^{3}H]diprenorphine and [^{11}C]carfentanil was significantly reduced in the endosomal environment compared to the extracellular (*P*-values for [^{3}H]diprenorphine and [^{11}C]carfentanil = $P<0.001$ and $P<0.050$, respectively; Table 1).

• Receptor affinity was not significantly effected by changes in cellular environment for [^{3}H]diprenorphine (Table 1). Hence, changes in BP are potentially reflective of a decrease in receptor availability.

• A trend was seen for a reduced affinity in the endosomal environment compared to the extracellular for [^{11}C]carfentanil. Changes in BP in the different cellular environments could therefore reflect a change in both B_{max} and affinity.

Table 1. Comparison of Effect of Different Buffer on Opioid Receptor Binding

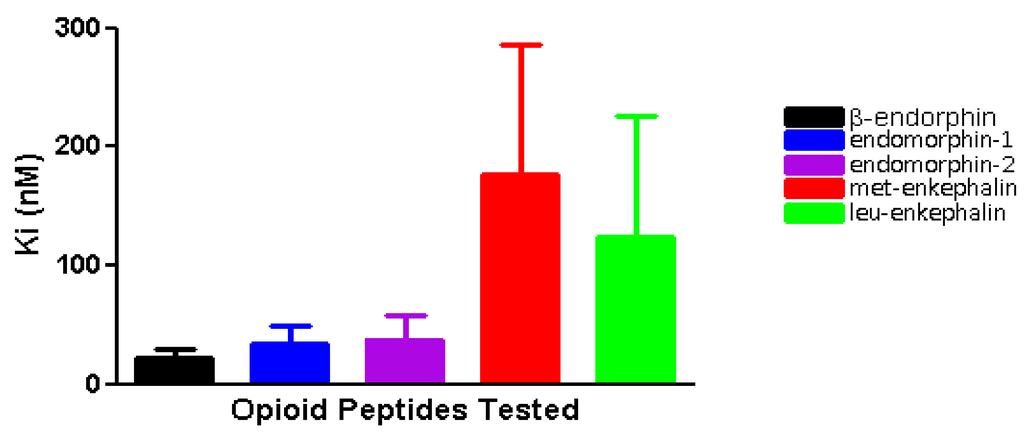
Ligand	Condition	B_{max} (fmol/mg protein)	B_{max} (pmol/g tissue)	K_d (nM)	*In vitro* BP
[^{3}H]Diprenorphine (μ-antagonist, δ-antagonist, κ-partial agonist)	Extracellular	286 ± 23	10.4 ± 1.08	0.33 ± 0.02	35 ± 2
	Intracellular	262 ± 20	9.53 ± 0.71	0.27 ± 0.01	36 ± 3#
	Endosomal	127 ± 27*	5.33 ± 1.17	0.36 ± 0.05	17 ± 1*
[^{11}C]Carfentanil (μ-agonist)	Extracellular	81 ± 11	7.07 ± 3.69	0.32 ± 0.13	20 ± 8
	Intracellular	67 ± 16	6.52 ± 3.70	0.79 ± 0.68	16 ± 9
	Endosomal	53 ± 10*	5.41 ± 2.45	0.77 ± 0.46	10 ± 3

*Mean ± SEM taken from 4 separate experiments for [^{3}H]diprenorphine and [^{11}C]carfentanil . Data analysis performed using GraphPad PRISM version 5.0; statistics using SigmaStat one-way ANOVA and Tukey post-test. *Represent comparisons between extracellular and endosomal and #represent comparisons between intracellular and endosomal (p-values for [^{3}H]diprenorphine and [^{11}C]carfentanil = P<0.001 and P<0.050, respectively). pmol/g tissue equivalent to nM. BP=B_{max} (nM)/K_d (nM).*

•Following competition studies the rank order of binding affinities for the opioid peptides at the μ-OR was (Figure 3):

β-endorphin > endormorphin-1 > endomorphin-2 > met-enkephalin > leu-enkephalin

Figure 3. Affinity of Peptides for μ-OR using in the Extracellular Environment



Error bars represent ± SEM from 4 separate experiments. Data analysis performed using GraphPad PRISM version 5.0. All data fitted best to one binding site.

Conclusions

• Conversion of the derived B_{max} and affinity data to *in vitro* binding potentials indicate that an internalisation process may significantly contribute to the reduction in signal (BP) observed in endogenous release studies using [^{11}C]diprenorphine.

  ○For [^{11}C]carfentanil, this change may also be driven by a reduction in affinity of μ-OR for radiotracer in the endosomal environment, however, due to the reduced reproducibility of *in vitro* studies with short lived radioisotopes, significance was not reached ($P = 0.3$).

• Competition data suggest that during endogenous release studies, μ-OR selective peptides such as endormorphin-1/2 and β-endorphin will have greater affinity than other endogenously released peptides and could have a greater contribution to regional signal changes observed by direct competition or initiation of agonist-mediated internalisation processes than met- and leu-enkephalin.

  •***To conclude: These data suggest that for the OR tracers investigated, a change in BP would primarily be driven by a reduction in observed receptor availability in the endosomal environment.***

References

(1) Koepp *et al,* 1998. (2) Scott *et al*, 2007. (3) Laruelle, 2000. (4)Trapaideze *et al,* 2000. (5) Song and Marvizon, 2003. (6) Li *et al,* 2000. (7) Kramer *et al*, 2000.

Acknowledgements

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