Development of a high throughput SPA assay using a cloned kappa opioid receptor.

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Introduction

Opioid receptors have been shown to be capable of producing strong analgesic effects when activated by opiates [1,2] and their potential role in pain relief is being investigated. Opioid receptor classes have been identified by the pharmacology of a range of selective ligands, these have been named mu (μ), delta (δ), and kappa (κ).

Amersham Biosciences has developed a scintillation proximity assay (SPA) for high throughput screening using a κ-opioid receptor membrane prepared from cloned HEK-293 cells (SignalScreen, Amersham Biosciences UK Ltd. 6110558 200U). A range of ligands known to have a high affinity for the κ-opioid receptor was used to generate IC_{50} values in this assay.

Methods

1mg WGA coated polyvinyl toluene (PVT) SPA bead, 15μg membrane and [^{3}H]diprenorphine (DPN) (concentration as shown below), were incubated for 18 hours at room temperature. Non specific binding (NSB) was determined in the presence of 50μM unlabelled naloxone. All assays were performed in a total volume of 200μl assay buffer: 50mM tris-HCl, pH7.4.

For competition binding curves, a range of concentrations of β-endorphin was prepared using buffer. Naltrexone and U50-488 were prepared using DMSO; final concentration of DMSO in well was 2.5%.

Results

Saturation binding experiments were carried out to determine K_d and Bmax. A range of concentrations of [^{3}H]DPN was prepared in assay buffer to give final concentrations as shown in figure 1.

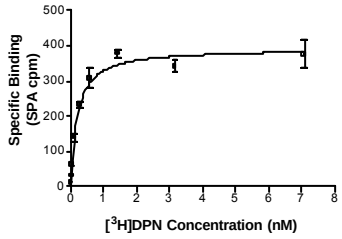

***Figure 1**. Results of saturation binding experiment. Values are means ±SD (n=3)*

Bmax and K_d were determined to be 1.99pmol/mg and 0.22nM respectively, in the SPA assay (experiments n=3), and were similar to those obtained in our experiments in filter binding assays: Bmax 1.75 pmol/mg, K_d 0.21nM (data not shown).

For high throughput screening purposes, a 96-well assay plate was used and the volume was therefore restricted to a maximum of 200μl. At [^{3}H]DPN concentration 0.2nM (K_d) the assay was shown to be in ligand depletion (>25% of added ligand was bound). In order to avoid this, and therefore accurately determine IC_{50} values, the ligand concentration was increased to 1.8nM. Under these conditions ligand depletion was <10%.

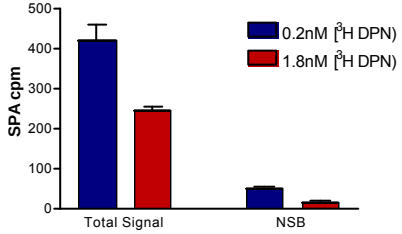

***Figure 2.** Total signal and NSB at [^{3}H]DPN concentrations of 0.2nM and 1.8nM*

Increasing [^{3}H]DPN concentration from 0.2nM to 1.8nM reduced the assay signal window (difference between total signal and NSB) from 17:1 to 9:1. The Z` factor[7] was calculated at the increased ligand concentration (1.8nM), this demonstrated that the assay was a good screening assay with a Z` of 0.76 (figure 3).

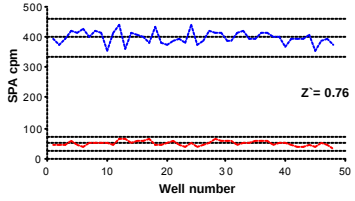

***Figure 3.** Z` factor analysis of SPA assay, total binding (·) NSB (·). Solid lines indicate mean of 48 observations, dotted lines indicate mean ± 3 x SD.*

The assay was also tested for tolerance to DMSO, a range of concentrations was included from 0-9% (v/v) (figure 4). The assay was tolerant to a DMSO concentration of at least 4.5% (v/v).

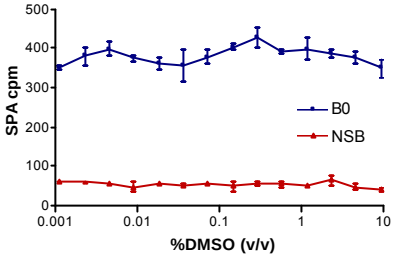

***Figure 4.** Total assay signal obtained at range of concentrations of DMSO from 0 - 9% (v/v). Values are means ±SD (n=3).*

Competition binding studies (figure 5) were performed using a range of ligands that have been shown to be selective for the κ-opioid receptor.[3, 4, 5]

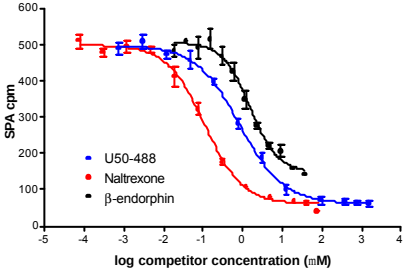

***Figure 5.** Binding of [^{3}H]DPN to k-opioid receptor captured with WGA coated PVT SPA beads. Competition with b-endorphin (·), naltrexone (·) and U50-488 (·). Values are means ±SEM (replicates n=3). IC_{50} values are shown in table 2.*

Ligand	IC_{50} (μM)	95% Confidence Intervals	Selective for subtype
U50-488	0.669	0.557-0.809	$?_1$ & $?_3$
Naltrexone	0.082	0.065-0.104	$?_1$
β-Endorphin	0.907	0.680-1.219	$?_2$

***Table 1.** IC_{50} values obtained for each subtype specific ligand tested (experiments n=3).*

CONCLUSIONS

- A high throughput-screening assay to identify compounds that bind to a cloned κ-opioid receptor has been developed.
- Tolerance to DMSO concentration up to at least 4.5% (v/v) has been demonstrated.
- Competition binding studies indicate that the ? subtype is predominantly expressed in this membrane preparation. This confirms previous studies that cloned κ-opioid receptors exhibit typical pharmacological K_1 profile.[5, 6]

References

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