CHARACTERISATION OF RADIO-lgAND BINDING BY SCINTILLATION PROXIMITY ASSAY TO ANGIOTENSIN II TYPE I, DOPAMINE D2, AND 5-HYDROXYTRYPTAMINE TYPE 1A RECEPTORS EXPRESSED IN INSECT CELLS USING THE BACULOVIRUS SYSTEM

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Introduction

Scintillation Proximity Assay (SPA) provides the researcher with a non-invasive technology and allows direct measurement of the radioactivity’s interaction with its target. One of the major advantages provided by this technology is the characterisation of receptor binding using wheat germ agglutinin (WGA)-coated scintillant-impregnated beads which bind to the glycosylated proteins in the receptor or membranes1,2. The baculovirus/insect cell expression system is a well established method for the production of functionally active receptors, such as the glutamate receptor3. SPA has previously been applied to studies on the soluble extracellular domain of the EGFR receptor expressed and glycosylated in Sf9 cells4. The aim of this study was to determine the feasibility of applying SPA technology to the S9 expression methodology. At direct contact with the additional filtration approach, using different receptor systems as examples, was conducted.

Methods

The receptor assay format and buffer conditions followed that recommended by BioSignal wherever possible. A standard membrane preparation from bovine adrenal cortex (BAC) was the source of the Angiotensin II type 1 receptor (SPA kit; Amersham NBC01). All other cloned receptor preparations were expressed in Sf9 cells and were from BioSignal. [3H]Angiotensin II (Amersham IM 248) was incubated at 27°C for 2.5 hours followed by 1.5 hours at room temperature with 28 μg (BAC) or 4 μg (Sf9) protein in 50 mM Tris HCl buffer, pH 7.4, containing 10 mM MgSO4, 0.5 mM EDTA and 0.1% ascorbic acid. Non specific binding was determined with 100 μM cold angiotensin II.

Results and Discussion

The Ks values obtained in competition studies on [3H]Tyr4Angiotensin II binding are essentially the same for receptors expressed in Sf9 cells or on BAC membranes when the assay is performed by SPA or by the standard filtration method (Figure 1). The Ks values in the systems are consistent with literature values5. For haploidal, the Ks values (1.17-1.4 μM, Table 1) for competition with [3H]epiprodipe binding to D2 receptors are similar to the 0.6 μM value reported by BioSignal, but lower than the literature values. The results demonstrate that the values obtained by SPA parallel those obtained by filtration.

Saturation and Scatchard plots for [3H]Tyr4Angiotensin II binding to the human dopamine D2 receptor expressed in Sf9 cells measured by SPA and filtration. The results are representative of 3 separate determinations and the data expressed as mean ± S.D. The Ks values determined by filtration were 0.5 and 0.04 nM for Sf9 and BAC respectively. Specific binding is the difference of the means of these values. The Ks and Bmax values determined were used to calculate apparent Bmax and Bmax respectively for SPA and Bmax values determined by filtration for SPA and Bmax values determined by filtration.

References