Contents

IN VITRO RECEPTOR AUTORADIOGRAPHY

Sectioning

Frozen brains are trimmed with a razor blade and mounted in a cryostat chuck. Tissue sections are cut at a thickness of 20 µm using a cryostat (Bright OTF5000) and thaw mounted onto Superfrost® slides. Three consecutive sections are placed on each slide with a total of three slides (9 sections) from each brain region. Slides are stored dessicated at -80 ^{O}C.

Incubation in radiotracer

Slides are warmed to room temperature whilst still in the slide box and then placed in pre-incubation buffer (50 mM Tris, 5 mM $MgCl_2$, 0.1 mM EDTA protease inhibitor cocktail, pH 7.4) for 30 minutes with gentle agitation. Slides are then removed from the buffer and are placed horizontally in a humidified box and 1 ml of the radioligand in assay buffer layered over each slide. Sections are incubated in the radioligand solution for 90 minutes at room temperature with periodic agitation. The radioligand solution is then rapidly aspirated off and the slides immediately placed in ice-cold wash solution (e.g. three washes, 5 minutes each). Following the final wash, the slides are dipped briefly in distilled water and dried under a stream of warm air.

Phosphorimaging

After drying, sections are placed over a multipurpose (^{125}I) or tritium-sensitive (^{3}H) phosphor screen together with autoradiographic standards. The screen is exposed for 1 - 7 days and then scanned on a phosphorimager (Cyclone Storage Phosphor System).

Data analysis

Regions-of-interest (ROIs) are drawn over each section. Radiotracer binding is determined in units of DLU/mm^2, converted to DPM/mm^2 by reference to autoradiographic standards. A value for specific binding is generated by the subtraction of mean nonspecific binding from mean total binding for each brain region. The phosphorimager (.bvr) images are exported as high resolution tiff files.

EX VIVO RECEPTOR OCCUPANCY DETERMINATION

Sectioning

Frozen brains or tissues from drug-treated animals are trimmed with a razor blade and mounted in a cryostat chuck. Tissue sections are cut at a thickness of 20 µm using a cryostat (Bright OTF5000) and thaw mounted onto Superfrost® slides. Three consecutive sections are placed on each slide, with a total of two slides (6 sections) per tissue.

Incubation in radiotracer

Slides are placed horizontally in a humidified box and radioligand in assay buffer (1 ml) layered over each slide. Sections are incubated in the radioligand solution for 5 - 10 minutes at room temperature. The radioligand solution is then rapidly aspirated off and the slides immediately placed in ice-cold wash solution (e.g. three washes, 5 minutes each). Following the final wash, the slides are dipped briefly in distilled water and dried under a stream of warm air.

Phosphorimaging

Sections are placed over a multipurpose (^{125}I) or tritium-sensitive (^{3}H) phosphor screen together with autoradiographic standards. The screen is exposed for 1 - 7 days and then scanned on a phosphorimager (Cylone Storage Phosphor System).

Data analysis

Regions-of-interest (ROIs) are drawn over each section and radioactivity levels measured in units of digital light units / mm^2 (DLU/mm^2). The DLU/mm^2 values are converted to CPM/mm^2 by reference to the autoradiographic standards. A value for specific binding is generated by the subtraction of mean nonspecific binding from mean total binding for each brain or organ. Percent inhibition of specific binding is plotted against the drug dose or plasma or tissue drug concentration to determine receptor occupancy.