Contents

IN VITRO AUTORADIOGRAPHY

Sectioning

Frozen brains 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 (Lieca® CM3600) 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 min 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 min at room temperature with periodic agitation. The radioligand solution is then rapidly aspirated off and the slides immediately placed in ice-cold wash solution (three washes, 5 min 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 - 5 days and then scanned on a Fujifilm® FLA-7000 scanner.

Data analysis

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

EX VIVO AUTORADIOGRAPHY

Sectioning

Animals previously treated with the radiolabeled drug (i.v.) are euthanized and the organs dissected free and snap-frozen in a dry-ice hexane slurry. Blood is collected into a collection vial at the time of sacrifice for subsequent analysis of plasma radioactivity levels. The frozen radioactive organs are sectioned at a thickness of 20 µm using a cryostat (Lieca® CM3600) and the sections thaw mounted onto Superfrost® slides. Three consecutive sections are placed on each slide, with a total of five slides (15 sections) per organ.

Phosphorimaging

After drying, sections are placed over a multipurpose (^{125}I) or tritium-sensitive (^{3}H) phosphor screen. The screen is exposed for 5 - 7 days and then scanned on a Fujifilm® FLA-7000 scanner.

Data analysis

Regions-of-interest (ROIs) are drawn over each section. Radiotracer binding is determined in units of psl/mm^2, converted to DPM/mm^2 by reference to autoradiographic standards placed on each screen and exported to an excel file. The DPM/mm^2 values for each organ are subsequently converted to % injected dose/g by reference to the section thickness and amount of radioactivity administered to each animal.

EX VIVO RECEPTOR OCCUPANCY

Sectioning

Frozen brains 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 (Lieca® CM3600) and thaw mounted onto Superfrost® slides. Three consecutive sections are placed on each slide, with a total of two slides (6 sections) per brain.

Incubation in radiotracer

Slides are placed horizontally in a humidified box and 1 ml of radioligand in assay buffer layered over each slide. Sections are incubated in the radioligand solution for 15 min at room temperature. The radioligand solution is then rapidly aspirated off and the slides immediately placed in ice-cold wash solution (three washes, 5 min 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 - 5 days and then scanned on a Fujifilm® FLA-7000 scanner.

Data analysis

Regions-of-interest (ROIs) are drawn over each section and radioactivity levels measured in units of psl/mm2. The psl/mm^2 values are converted to D.P.M/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.