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Use of LC/MS to assess brain tracer distribution in preclinical, in vivo receptor occupancy studies: Dopamine D2, serotonin 2A and NK-1 receptors as examples

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Abstract

High performance liquid chromatography combined with either single quad or triple quad mass spectral detectors (LC/MS) was used to measure the brain distribution of receptor occupancy tracers targeting dopamine D2, serotonin 5-HT2A and neurokinin NK-1 receptors using the ligands raclopride, MDL-100907 and GR205171, respectively. All three non-radiolabeled tracer molecules were easily detectable in discrete rat brain areas after intravenous doses of 3, 3 and 30 μg/kg, respectively. These levels showed a differential brain distribution caused by differences in receptor density, as demonstrated by the observation that pretreatment with compounds that occupy these receptors reduced this differential distribution in a dose-dependent manner. Intravenous, subcutaneous and oral dose–occupancy curves were generated for haloperidol at the dopamine D2 receptor as were oral curves for the antipsychotic drugs olanzapine and clozapine. In vivo dose–occupancy curves were also generated for orally administered clozapine, olanzapine and haloperidol at the cortical 5-HT2A binding site. In vivo occupancy at the striatal neurokinin NK-1 binding site by various doses of orally administered MK-869 was also measured. Our results demonstrate the utility of LC/MS to quantify tracer distribution in preclinical brain receptor occupancy studies.

Keywords: Dopamine; D2; Serotonin; 5-HT2A; Neurokinin; NK-1; Receptor occupancy; In vivo binding; Liquid chromatography; Mass spectroscopy; Haloperidol; Clozapine; Olanzapine; MK-869; Raclopride; MDL-100907; GR205171; Rat; Gerbil

Introduction

Brain receptor occupancy is measured in the clinic using radioisotopically labeled tracer ligands whose distribution in the brain can be determined by non-invasive methods, often involving imaging. The most common of these methods are positron emission tomography and single photon emission computed tomography. Both of these techniques employ radioisotope-labeled receptor ligands which are injected, in low "tracer" doses, into humans where their interaction with target receptors can be assessed by the kinetics of their distribution. In preclinical studies, the localization of these tracer ligands in brain regions is most often determined through tissue dissection and subsequent scintillation spectroscopy (Zhang and Bymaster, 1999; Wadenberg et al., 2000; Stockmeier et al., 1993). In all the above cases, the tracer ligand used to probe brain receptor occupancy is radiolabeled, thought to be necessary because of the low doses used and subsequent need to measure very low compound levels in tissues. We have found that the localization of tracer molecules in the preclinical animal setting can be accomplished using liquid chromatography coupled to mass spectroscopy (LC/MS), obviating the need for radiolabeled materials.

The use of LC/MS to measure tracer levels in discrete brain areas and thereby assess the brain receptor occupancy of CNS drugs and drug candidates confers a number of advantages. First, it removes the need to use radiolabeled tracers with their associated expenses, both financial and environmental. Second, it greatly enhances the speed at which new tracer candidates can be evaluated in vivo, eliminating the need to radiolabel

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candidate molecules. Third, with LC/MS, the tracer of interest is quantified with no contribution from radiolabeled metabolites. Finally, it is possible to measure, in a single sample, multiple tracers plus the compound for which the occupancy is being assessed, e.g. the blocking compound. This allows the generation of dose–occupancy curves as well as brain concentration–occupancy curves for a given test compound, the latter being independent of pharmacokinetic linearity. The use of LC/MS in receptor occupancy studies facilitates their routine use, and even more importantly, the discovery of new tracers for novel receptor targets.

In vivo receptor occupancy can add critical information to a drug discovery or basic research effort. This technique facilitates the rapid development of new tracers and assesses the in vivo binding of a drug candidate to the target receptor. Receptor occupancy data can help clarify the association of behavior, physiology or neurochemistry with a specific neuronal receptor mechanism and add greatly to the validation state of a drug discovery target.

Materials and methods

Animals

Adult male Sprague–Dawley rats (250 to 320 g, HSD, Indianapolis, IN) were used for the dopamine D2 (D2) and serotonin 2A (5-HT2A) receptor occupancy studies. Rats were housed 6 per cage in standard rack housing. Food and water were available ad libitum except where noted below. Mongolian gerbils were used for the neurokinin 1 (NK-1) receptor occupancy studies because of species differences between human and rodent receptors (Gitter et al., 1991). Male gerbils, weighing between 30 and 35 g, were obtained from Charles River Laboratories, Wilmington, NC.

Compounds

The word "tracer" refers to ligands that are administered at low doses to characterize receptor occupancy at a specific target. Raclopride was purchased from Sigma-Aldrich, St Louis, MO and used as a receptor occupancy tracer for D2 sites in the brain (Kohler et al., 1985). It was dissolved in saline and administered intravenously at a dose of 3 μg/kg. MDL-100907 (synthesized at Eli Lilly and Company) was used as a tracer for 5-HT2A sites and was dissolved in water and administered intravenously at a dose of 3 μg/kg. GR205171 (synthesized at Eli Lilly and Company) was used as a receptor occupancy tracer for NK-1 receptors (Bergstrom et al., 2000). It was administered intravenously in saline at a dose of 30 μg/kg. For rats, tracers were administered intravenously into the lateral tail vein during brief restraint. For NK-1 experiments employing gerbils, animals were briefly anesthetized with isoflurane, the skin overlying the femoral vein was cut and retracted and an intravenous injection was administered into the femoral vein. After the injection, the wound was closed and the gerbils were immediately allowed to recover from anesthesia. Animals were sacrificed 10, 15 and 30 min after tracer administration for the D2, 5-HT2A and NK-1 receptor occupancy assays, respectively.

"Test compound" refers to compounds for which brain receptor occupancy was assessed using the receptor occupancy tracers listed above. Haloperidol and clozapine were purchased from Sigma-Aldrich. Olanzapine and MK869 were synthesized at Eli Lilly and Company. Drugs were solubilized in acidified water, and the solution pH was subsequently increased to values between 5 and 7. Test compounds were dosed intravenously, subcutaneously or orally by gavage 15, 30 or 60 min prior to intravenous tracer administration, respectively.

Tissue preparation and analysis

Rats or gerbils were sacrificed by cervical dislocation or decapitation, and the brain was quickly removed, dissected, and placed in small centrifuge tubes on ice. For raclopride and GR205171 studies, the striatum (bilateral) and cerebellum were collected. For MDL-100907, frontal cortex and cerebellum were used. For raclopride and MDL-100907 analysis, the tissue was homogenized, using an ultrasonic dismembrator probe (Fisher Scientific model 100, Pittsburgh, PA), in four volumes (W/V) of acetonitrile containing 0.1% formic acid and centrifuged at 20,000 $\times g$ for 14 min in order to extract the tracer. For the GR205171 assay, samples of cerebellum and striatum were homogenized in 400 and 200 μl of the acetonitrile solution, respectively, and then centrifuged as described above. An aliquot of supernatant containing the tracer was diluted in water to an acetonitrile content less than that of the mobile phase and 10 μl was injected by autosampler onto an HPLC employing a Zorbax SB-C18 column (part# 866953-902, Agilent Technologies, Wilmington, DE). Separation was achieved using isocratic conditions and a mobile phase of water/acetonitrile containing 0.1% formic acid with a flow rate of either 0.5 mL/min (raclopride) or 0.35 mL/min (MDL-100907 and GR205171). For the raclopride, MDL-100907 and GR205171 analysis, the percents acetonitrile used in the mobile phase were 45%, 50% and 55%, respectively. For the raclopride assay, compound eluting from the column was measured using an Agilent model 1946A single quad mass spectrometer set to selectively monitor the mass-to-charge ratio of singly protonated raclopride (347.1). GR205171 and MDL-100907 were quantified after elution from the HPLC using an API 3000 LC/MS/MS mass spectrometer (Applied Biosystems, Foster City, CA) in positive electrospray mode using MRM methods and monitoring 433.2/160.1 and 374.2/204.2 ion pairs, respectively. Chromatographic assays were calibrated using standard curves generated by extracting a series of brain tissue samples from non-treated animals to which known quantities of analyte had been added.

Occupancy measures

Receptor occupancy was assessed using the well-established ratio method (Wadenberg et al., 2000), where the level of tracer in an area of high receptor density is "normalized" by that in an area of very low or absent receptor density. In the case of the D2