SUPPLEMENTAL DATA

Western Blot

Proteins were extracted from cultured cell suspensions. Cells were washed twice with cold PBS and resuspended in RIPA cell lysis buffer containing protease inhibitor mix P8340 (Sigma Aldrich, St. Louis, USA) for 30 min at 4°C. Lysed cells were centrifuged at 20000 g for 20 min at 4°C in order to discard any remaining cell debris. Supernatant containing proteins was collected and stored at -20°C until use. The amount of protein present in each sample was quantified using a BCA Protein Assay (Pierce, Rockford, IL, USA). For Western blot analysis, denaturated (95°C, 5 min) proteins (10 µg) were separated by SDS-PAGE before transfer to an Amersham Hybond PVDF membrane (GE Healthcare Life Sciences, Buckinghamshire, UK). Membranes were pre-saturated for two hours at room temperature with a 0.2% PBS-TWEEN 20 (PBST) solution containing either 3% of BSA or 3% of skim milk. Membranes were incubated overnight at 4°C with the primary antibody diluted in PBST containing 3% of BSA for the rabbit anti-rat TSPO antibody NP155 (provided by Dr. M. Higuchi, NIRS, Japan; 1/10000 dilution) and the rabbit anti-caspase3 primary antibody (Cell Signaling Technology, Beverly, MA; # 9665; 1/500) or in 3% skim milk for the mouse anti-tubulin primary antibody (Sigma Aldrich; # TG074; 1/20000). After three 10-min wash sessions in PBST, the membranes were incubated for 1 h at room temperature with the appropriate secondary horseradish peroxidase coupled antibodies (Santa Cruz Biotechnology; Santa Cruz; CA; 1/2,000) in PBST dilution (1/2000) containing 1% of BSA or 1% of skim milk. After three 10-min wash sessions, the membranes were developed using an Amersham ECL Western Blotting System (GE Healthcare Life Sciences) onto films.

Radiochemistry

Ready-to-inject, >99% radiochemically pure [^{18}F]DPA-714 (*N,N*-diethyl-2-(2-(4-(2-[^{18}F]fluoroethoxy)phenyl)-5,7-dimethylpyrazolo[1,5-a]pyrimidin-3-yl)acetamide) was prepared from cyclotron-produced [^{18}F]fluoride (Cyclone-18/9 cyclotron, IBA, Louvain-la Neuve, Belgium) using a commercially available TRACERLab™ FX-FN synthesizer (GEMS, Buc, France), a process which was formerly performed on a Zymate-XP robotic system (Zymark, Hopkinton (MA), USA). Radiolabelling of DPA-714 with fluorine-18 (half-life 109.8 min) uses a tosyloxy-for-fluorine nucleophilic aliphatic substitution (one-step process) and its preparation includes the following five steps : (1) dilution of the no-carrier-added, dried (activated) K[^{18}F]F-Kryptofix®222 complex (prepared from [^{18}F]fluoride, potassium carbonate and Kryptofix®222) with 700 µL of dimethylsulfoxide containing 3.5 to 4.5 mg of the tosyloxy precursor for labeling (*N,N*-diethyl-2-(2-(4-(2-toluenesulfonyloxyethoxy)phenyl)-5,7-dimethylpyrazolo[1,5-a]pyrimidin-3-yl)acetamide); (2) heating the reaction mixture at 160°C for 5 min; (3) dilution of the reaction mixture with the high performance liquid chromatography (HPLC) mobile phase and pre-purification on a SepPak® Alumina N™ cartridge; (4) HPLC purification on a semi-preparative Waters X-Terra™ C-18 column (eluent 0.1M aq. ammonium acetate (pH10) / acetonitrile : 60 / 40 (v:v)); and (5) SepPak® Plus C-18 cartridge-based removal of the HPLC solvents. [^{18}F]DPA-714, as an ethanolic (15%) physiological saline (aq. 0.9% NaCl) solution (6.7 to 8.5 GBq batches, 10 mL-volume), is routinely obtained within 50-55 min starting from 35 GBq of [^{18}F]fluoride (19-24% non-decay-corrected overall isolated yields) with specific radioactivities ranging from 41 to 244 GBq/µmol. Quality controls were performed on an aliquot of the ready-to-inject [^{18}F]DPA-714 preparation, in compliance with our in-house quality control/assurance specifications.

Immunohistochemistry

Brain sections were first fixed in 4% paraformaldehyde (PFA) for 15 min at room temperature (RT). To block PFA, NH_4Cl (50 mmol/L) in PBS was used for 5 min at RT. Tissue permeabilization was performed using methanol-acetone at a 1:1 volume ratio (-20°C, 5 min) followed by Triton 0.1% in PBS for 5 min at RT. Finally, sections were washed with PBS. For immunohistochemical labeling, these sections were saturated with a solution of bovine serum albumin 5% / Tween 0.5% in PBS for 15 min at RT to minimize nonspecific binding. The sections were then incubated for 1 hour at RT in a humidified chamber with primary antibodies in bovine serum albumin 5% / Tween 0.5% in PBS. Adjacent sections were stained with the following antibodies, a rabbit anti-rat TSPO (NP155, 1:1000), a mouse anti-rat CD11b (1:300, Serotec, Raleigh, NC, #MCA27514), and a chicken anti-rat glial fibrillary acidic protein (GFAP, 1:1000, Abcam, Cambridge, MA, #4674) [49]. The sections were washed for 3 min in PBS to remove the excess antibody and incubated for 30 min at RT with the appropriate secondary antibodies in bovine serum albumin 5% / Tween 0.5% in PBS: Alexa Fluor (AF)-488 goat anti-rabbit IgG (1:1000, #A11034 for the rat anti-TSPO antibody NP155), AF-594 goat anti-mouse IgG (1:1000, A11032 for mouse anti-rat CD11b antibody) and AF-647 nm goat anti-Chicken IgG (1/1000, #A21449 for the chicken anti-rat GFAP antibody). All secondary antibodies were purchased from Invitrogen (Paisley, UK). Negative controls were performed by omitting the primary antibody. Sections were washed again for 10 min (repeated three times) in PBS before being mounted with ProLong gold antifade reagent containing 4',6-diamidino-2-phenylindole (DAPI, Invitrogen P36931) in order to stain cell nuclei.

Autoradiography and Competitive Binding

[^{18}F]DPA-714 autoradiographic studies were performed using 20 μm brain sections. Adjacent cryosections were immersed in Tris Buffer (50mM TRIZMA preset crystals purchased from Sigma-Aldrich) adjusted to pH 7.4 with NaCl containing 49.4 MBq of [^{18}F]DPA-714 either alone (n=7) or in the presence of 20 μM unlabeled DPA-714 (n=7) for 20 min at 4°C. The unbound excess ligands were removed by two 2 min wash cycles in cold buffer and then a final rinse in cold deionized water. Sections were then placed in direct contact with a Phosphor-Imager screen (Molecular Dynamics, Sunnyvale, CA) and exposed overnight.