

Radiosynthesis and initial evaluation of [^{18}F]-FEPPA for PET imaging of peripheral benzodiazepine receptors

Alan A. Wilson^{a,b,*}, Armando Garcia^a, Jun Parkes^a, Patrick McCormick^{a,c},
Karin A. Stephenson^a, Sylvain Houle^{a,b}, Neil Vasdev^{a,b}

^aPET Centre, Centre for Addiction and Mental Health, Toronto, Ontario, Canada M5T 1R8

^bDepartment of Psychiatry, University of Toronto, Toronto, Ontario, Canada M5T 1R8

^cInstitute of Medical Science, University of Toronto, Toronto, Ontario, Canada M5S 1A8

Received 9 November 2007; received in revised form 13 December 2007; accepted 17 December 2007

Abstract

Introduction: A novel [^{18}F]-radiolabelled phenoxyanilide, [^{18}F]-FEPPA, has been synthesized and evaluated, in vitro and ex vivo, as a potential positron emission tomography imaging agent for the peripheral benzodiazepine receptor (PBR).

Methods: [^{18}F]-FEPPA and two other radiotracers for imaging PBR, namely [^{11}C]-PBR28 and [^{11}C]-PBR28-*d*3, were synthesised and evaluated in vitro and ex vivo as potential PBR imaging agents.

Results: [^{18}F]-FEPPA is efficiently prepared in one step from its tosylate precursor and [^{18}F]-fluoride in high radiochemical yields and at high specific activity. FEPPA displayed a K_i of 0.07 nM for PBR in rat mitochondrial membrane preparations and a suitable lipophilicity for brain penetration (log *P* of 2.99 at pH 7.4). Upon intravenous injection into rats, [^{18}F]-FEPPA showed moderate brain uptake [standard uptake value (SUV) of 0.6 at 5 min] and a slow washout (SUV of 0.35 after 60 min). Highest uptake of radioactivity was seen in the hypothalamus and olfactory bulb, regions previously reported to be enriched in PBR in rat brain. Analysis of plasma and brain extracts demonstrated that [^{18}F]-FEPPA was rapidly metabolized, but no lipophilic metabolites were observed in either preparation and only 5% radioactive metabolites were present in brain tissue extracts. Blocking studies to determine the extent of specific binding of [^{18}F]-FEPPA in rat brain were problematic due to large perturbations in circulating radiotracer and the lack of a reference region.

Conclusions: Further evaluation of the potential of [^{18}F]-FEPPA will require the employment of rigorous kinetic models and/or appropriate animal models.

© 2008 Elsevier Inc. All rights reserved.

Keywords: Positron emission tomography; Peripheral benzodiazepine receptor; Rats; Microglia

1. Introduction

The peripheral benzodiazepine receptor (PBR), now also known as the translocator protein (18 kDa) [1] is widely recognized as an important target for imaging by positron emission tomography (PET) and single photon emission computed tomography. In the central nervous system (CNS), the predominant location of the PBR is on the outer membrane of mitochondria associated with microglial

cells, and increases in PBR are considered a marker for neuroinflammation via microglia activation [2–6]. The prototypical radiotracer used for PET imaging of PBRs is [^{11}C]-PK11195 [7–11]; however, this radiotracer is reported to have several limitations as a PBR imaging tool, including high nonspecific binding, low brain penetration, high plasma protein binding and difficult preparation. Although some of these limitations may be specious, overall better PET radiotracers for imaging PBRs would be desirable. Considerable efforts have recently been made in this direction [12]. Promising candidates include FG1N-1-27 and analogues [13], DPA 713 [14,15], DAA1106 [16–18], FEDAA1106 [19] and PBR28 [20,21], the last three progressing to human PET studies [22–24] (Fig. 1).

* Corresponding author. PET Centre, Centre for Addiction and Mental Health, Toronto, Ontario, Canada M5T 1R8. Tel.: +1 416 979 4286; fax: +1 416 979 4656.

E-mail address: alan.wilson@camhpet.ca (A.A. Wilson).

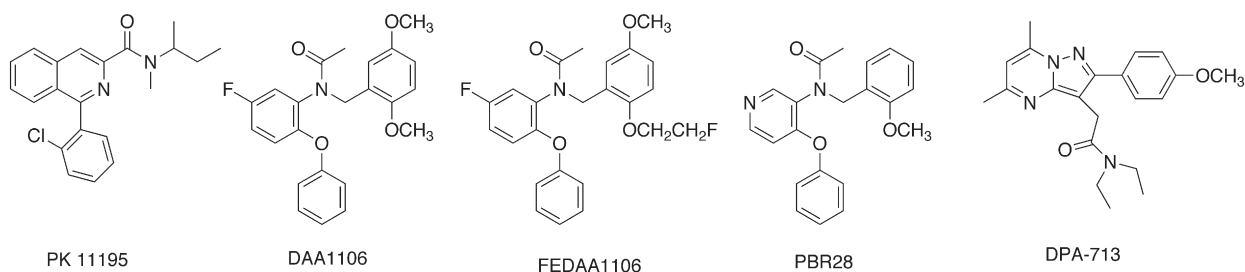


Fig. 1. Examples of radiotracers for imaging PBR using PET.

For our own inhouse imaging programme for PBR imaging, we were especially attracted to PBR28. This aryloxyanilide is a lean analogue of DAA1106, with a pyridine ring replacing one of the benzene rings of DAA1106. This clever strategy results in a compound in which affinity for PBR is retained as is blood–brain barrier penetration, lipophilicity is also reduced and pharmacokinetics in nonhuman primates and humans is improved [24,25]. Its radiosynthesis is also straightforward. However, we were concerned that early reports suggested that a significant amount of radioactivity in the brain following injection of PBR28 might originate from a radiolabelled metabolite [20,25]. Although this observation was in rats, were it carried over to humans, a problematic confound could result, limiting the quantification of PET PBR imaging using this radiotracer.

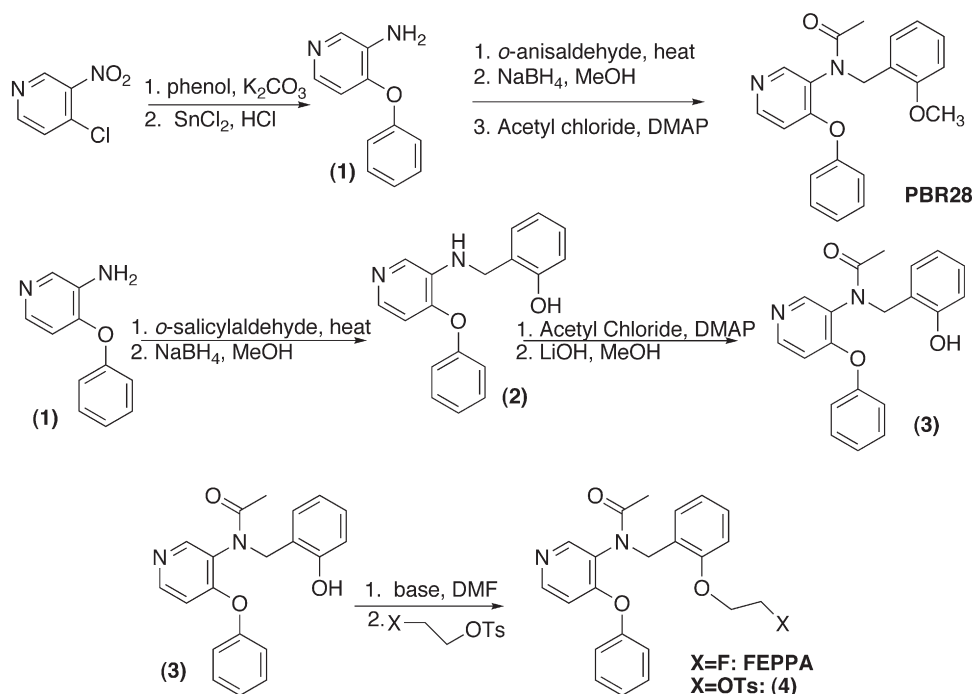
We report here the radiosynthesis and initial evaluation of an F-18 radiolabelled analogue of PBR28 (FEPPA, Scheme 1) and compare its *in vitro*, physiochemical and *in*

vivo CNS distribution properties, including metabolism in rats, to PBR28. Our results show that FEPPA has a very high affinity for PBR, has reduced metabolism and greater brain penetration compared to PBR28 in rats, is readily radiolabelled with [^{18}F] in high radiochemical yields and could be useful as a PET radiotracer for imaging PBR using PET.

2. Results and discussion

2.1. Chemistry

The synthesis of the target compounds (PBR28, FEPPA) and the precursors required for their radiolabelling (**3** and **4**) are shown in Scheme 1. The routes taken were generally those described in the literature [13,26–28]. We found, however, that condensation of the phenoxyaniline with the substituted benzaldehydes occurred most efficiently when effected as a melt rather than in methanol, as previously described [13].



Scheme 1. Synthetic route to precursors and standards.

Alkylation of the phenol (**3**) by ethyleneglycol bistosylate gave a complex mixture of products, which required careful chromatography to isolate the desired tosylate (**4**) at the high purity required for radiolabelling.

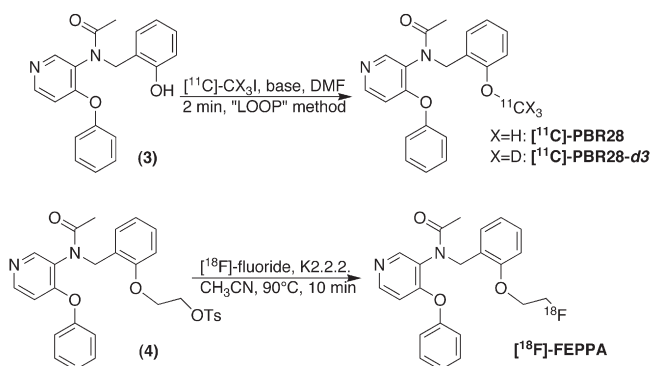
2.2. Radiochemistry

2.2.1. [^{11}C]-radiochemistry

As previously reported, [^{11}C]-PBR28 was efficiently and rapidly synthesized from its normethyl precursor (**3**) and [^{11}C]- CH_3I (Scheme 2) using the “LOOP” method [20,29]. By substituting LiAlD_4 for LiAlH_4 in the preparation of [^{11}C]- CH_3I , the isotopologue [^{11}C]PBR28-*d3* could be prepared in an identical manner [30]. In both cases, isolated radiochemical yields (uncorrected for decay) were 10–15% in a synthesis time of 30 min. Radiochemical purities were >98%, and specific activities were 1–2 Ci/ μmol at end of synthesis.

2.2.2. [^{18}F]-Radiochemistry

Incorporation of [^{18}F]-fluoride into the tosylate precursor (**4**) was both efficient and rapid under mild conditions (Scheme 2). While 5 min reaction at 90°C was usually sufficient, the reaction time was extended to 10 min as occasionally the reaction proceeded more slowly. Incorporation of [^{18}F]-fluoride was always >90%, reaching >98% upon occasions. [^{18}F]-fluoride was trapped on a small ion-exchange resin; a 4% water in acetonitrile solution of $\text{K}_2\text{CO}_3/\text{Kryptofix222}$ was used to elute the [^{18}F]-fluoride, as described by Gomzina [31]. With this method, azeotropic distillation with additional acetonitrile was not required to produce reactive [^{18}F]-fluoride. After high-performance liquid chromatography (HPLC) purification of the reaction mixture, isolated radiochemical yields of the final formulated product of 50–60% (uncorrected for decay) were obtained in a synthesis time of 40–50 min. Specific activities (at end of synthesis) ranged from 1.2 to 2.7 Ci/ μmol , and radiochemical purities of [^{18}F]-FEPPA were >99%. Coinjection of the radioactive product with an authentic standard of FEPPA under different conditions (solvents, pH, wavelength) with different analytical HPLC columns (Waters, Alltech, Phenomenex) further established the identity of the radiotracer.



Scheme 2. Synthesis of radiotracers.

Table 1

Comparison of affinities and lipophilicities of test compounds in competition with [^3H]-PK 11195 in rat mitochondrial fractions

Compound	K_i (nM)	Log D ^a
PK 11195	1.29	4.0
DPA 713	0.87	2.41 ^b
PBR 28	0.22	2.68
FEPPA	0.07	2.99

^a Measured between octanol and phosphate buffer at pH 7.4.

^b Measured by HPLC [14].

Under all conditions, [^{18}F]-FEPPA cochromatographed with authentic FEPPA. The formulated radiotracer displayed no radiolysis for at least 120 min post formulation and required no stabilizing agents such as ascorbate.

2.3. Physicochemical properties

The affinity of FEPPA for PBRs was measured by competition with [^3H]PK11195 in rat mitochondrial membranes. Three other compounds with affinity for PBR were also tested for comparison purposes (Table 1). In this assay, FEPPA was threefold more potent than PBR28 and an order of magnitude more potent than either DPA 713 or the prototypical PK 11195. Octanol-buffer partition coefficient measurement [32] gave a log D of 2.99, slightly higher than PBR28 (2.68), as would have been predicted from the extension of the methoxy-substituent to the larger 2-fluoroethoxy-substituent [33].

2.4. Metabolism studies

In order to compare the extent of metabolism of [^{18}F]-FEPPA with [^{11}C]-PBR28, rats were injected with the radiotracers, and plasma and brain extracts were isolated. HPLC analysis of radioactivity in rat plasma showed that both radiotracers were rapidly metabolized with only 7–20% parent compound remaining in plasma 40 min post injection (Table 2). At all time points examined, the extent of metabolism was greater for [^{18}F]-FEPPA than [^{11}C]-PBR28. HPLC analysis of brain extracts confirmed the findings of Briard et al. [20] in that about 10–15% of radioactivity in rat brain following [^{11}C]-PBR28 injection was due to radioactive metabolites. The percentage of radioactivity in

Table 2

Results from analysis of metabolites of [^{11}C]-PBR 28, [^{11}C]-PBR28-*d3* and [^{18}F]-FEPPA in rat brain and plasma at various times post radiotracer injection

Time	% Unmetabolized [^{11}C]-PBR28		% Unmetabolized [^{11}C]-PBR28- <i>d3</i>		% Unmetabolized [^{18}F]-FEPPA	
	Plasma	Brain	Plasma	Brain	Plasma	Brain
control	>99	>99.5	>99	>99	97.0	>99.5
5 min	53.5	98.1	37.6	98.5	33.1	98.5
15 min	50.7	91.9	—	—	11.9	95.5
40 min	20.7	84.6, 84.8	20.8	89.5, 90.5	7.2	93.3, 95.4

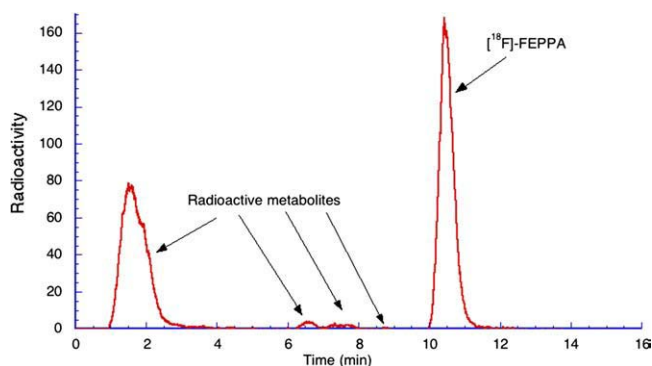


Fig. 2. HPLC radiochromatogram of rat plasma 5 min post injection of the radiotracer $[^{18}\text{F}]\text{-FEPPA}$.

rat brain extracts derived from $[^{18}\text{F}]\text{-FEPPA}$ metabolism was less at only 5%. One approach to reducing rates of metabolism of radiotracers is the introduction of deuterium in judicious positions of the molecule [34–36]. To this end, the isotopologue $[^{11}\text{C}]\text{-PBR28-}d_3$ was synthesized and similarly analyzed. However, no significant differences were observed between the isotopologues either in plasma or brain extracts (Table 2). In no case, in plasma or in brain extracts, was any lipophilic metabolite species detected deriving from any of the three radiotracers. A typical HPLC chromatogram of radioactivity in plasma following injection of $[^{18}\text{F}]\text{-FEPPA}$ is shown in Fig. 2.

2.4.1. Ex vivo biodistribution studies with $[^{18}\text{F}]\text{-FEPPA}$ in rats

Upon intravenous radiotracer administration, moderate levels of radioactivity were observed in rat brain (Fig. 3), indicating reasonable blood–brain barrier penetration of $[^{18}\text{F}]\text{-FEPPA}$. Standard uptake values at 5 min post injection were 0.6 in whole brain with a slow washout to 0.35 after 60 min post injection. Only a modest regional differentiation of radioactivity was observed, consistent with the ubiquitous distribution of PBR in rat brain [11,16,37]. Somewhat higher levels of radioactivity were observed in the olfactory bulb and hypothalamus, also consistent with the reported distributions of PBR in this species [11,16,37].

2.4.2. Attempts to measure the specific binding signal of $[^{18}\text{F}]\text{-FEPPA}$ and $[^{11}\text{C}]\text{-PBR28}$

A crucial step in the evaluation of a radiotracer for in vivo imaging of a target protein is the determination of the proportion of binding which is specific to the target, i.e., the specific-to-nonspecific ratio (SBR). A high level of non-specific binding is probably the most common hurdle that a prospective radiotracer fails to clear [38]. Most conveniently, SBRs are estimated by comparing levels of binding in target-rich regions to target-poor regions or target-devoid regions. With the assumptions that delivery is the same to both regions (usually true within the brain) and that nonspecific binding is the same in the examined regions (assumed to be

true), the SBR is simply given by the ratio of activity minus 1 in the prescribed regions. However, there is no reference region devoid of PBRs in rat brain, disqualifying such an approach. Estimation of the specificity of binding of a radiotracer can also be obtained by pre- or coinjecting a large amount of a drug known to bind to the target, thereby saturating the receptor preventing any specific binding of the administered radiotracer. This technique assumes that the blocking drug has no effect on delivery of radiotracer to the target, which is often untrue and is certainly untrue in the cases of $[^{18}\text{F}]\text{-FEPPA}$ and $[^{11}\text{C}]\text{-PBR28}$. Regional and total brain uptake of radioactivity following $[^{18}\text{F}]\text{-FEPPA}$ injection is only minimally effected by coadministration of a range of doses of PBR28 (Fig. 4, left). However, radioactivity levels in the plasma dramatically increase in a dose-dependant fashion. The effect is most clearly seen if the region to plasma ratios as a function of dose are plotted (Fig. 4, right). Nearly identical results are obtained when $[^{11}\text{C}]\text{PBR28}$ is employed as the radiotracer and FEPPA is used as the competing drug (Fig. 5). We have also found the same phenomenon when $[^{18}\text{F}]\text{-FEPPA}$ is coadministered with “cold” FEPPA or $[^{11}\text{C}]\text{-PBR28}$ is coadministered with cold PBR28 (results not shown).

The most straightforward explanation of such results is that the competing drug is binding to peripheral PBR sites, thus increasing the amount of radiotracer in plasma and, concomitantly, the amount available to penetrate the brain. Increased brain delivery of a radiotracer by blockade of peripheral binding sites has been reported for several radiotracers including SERT (serotonin transporter) ligands [39]. In a study of the PBR radiotracers PBR01 and PBR06, Imaizumi et al. [40] recently reported large (fivefold) increases in plasma levels and two- to threefold increases in initial brain uptake following administration of competing drug. It is not possible to determine the amount of specific binding of either $[^{18}\text{F}]\text{-FEPPA}$ or $[^{11}\text{C}]\text{-PBR28}$ in rat brain using only the data presented here unless gross assumptions

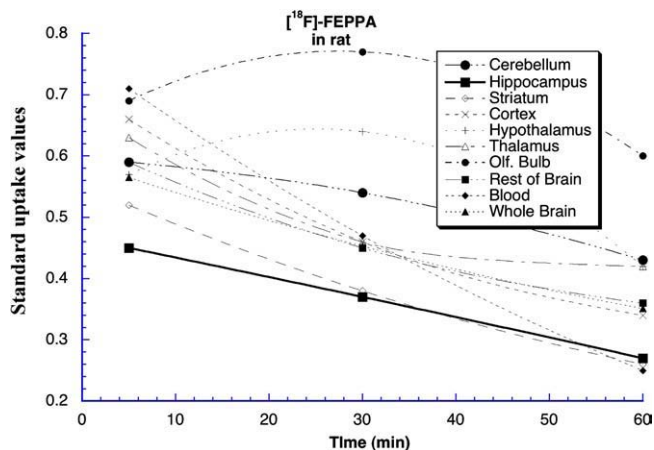


Fig. 3. Rat brain biodistribution of $[^{18}\text{F}]\text{-FEPPA}$. Error bars omitted for clarity; standard deviations were <10% except for Olf. Bulb (ca. 20%).

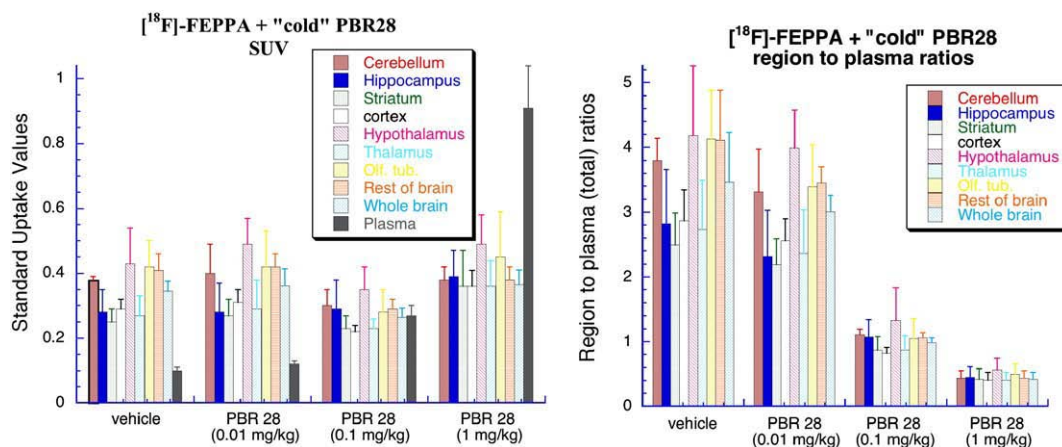


Fig. 4. (Left) Effect of coadministering various doses of PBR28 with [¹⁸F]-FEPPA on the regional rat brain and plasma levels of radioactivity. (Right) Results expressed as ratios of radioactivity in rat brain region to plasma.

are invoked (see Ref. [41]). Full kinetic modelling, with a metabolite-corrected arterial input function, will be required to determine the exact extent of specific binding. Such PET measurements in a larger species (pigs) are proceeding.

3. Conclusions

Further examination of [¹⁸F]-FEPPA as a PBR PET imaging radiotracer is warranted for several reasons. It has subnanomolar affinity for PBR *in vitro*, is readily synthesized in high quantity and quality, penetrates the rat blood-brain barrier and displays few radioactive metabolites in rat brain. However, the *in vivo* sensitivity and specificity of [¹⁸F]-FEPPA binding to PBR is undetermined, and further evaluation using more sophisticated animal models will be required to evaluate its potential. Either full kinetic modelling with an accurate input function and/or evaluation in a validated animal model of PBR expression will be required.

4. Experimental methods

4.1. General

A Scanditronix MC 17 cyclotron was used for radio-nuclide production. Purifications and analyses of radioactive mixtures were performed by HPLC with an inline UV (254 nm) detector in series with an NaI crystal radioactivity detector (radiosynthesis and quality control [QC]) or a Bioscan Flowcount coincidence radioactivity detector (metabolite analysis and QC). Isolated radiochemical yields were determined with a dose calibrator (Capintec CRC-712M). Chemicals were obtained from Aldrich, Alpha Aesar or Fisher and used as supplied unless otherwise noted. Tetrahydrofuran was freshly distilled under nitrogen from LiAlH₄, while dimethylformamide (DMF) was distilled from barium oxide at reduced pressure and stored over 4Å molecular sieves prior to use. Acetonitrile was stored over 4Å molecular sieves prior to use. Radiosyntheses were

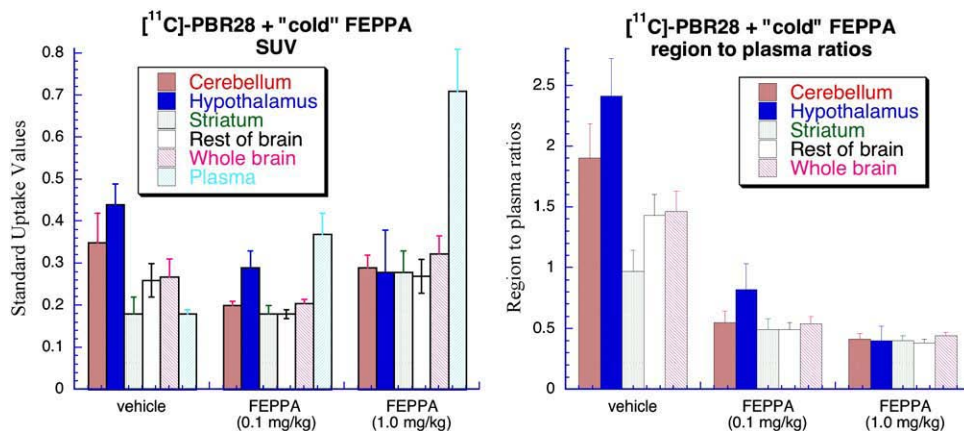


Fig. 5. (Left) Effect of coadministering various doses FEPPA with [¹¹C]-PBR28 on the regional rat brain and plasma levels of radioactivity. (Right) Results expressed as ratios of radioactivity in rat brain region to plasma.

performed using a robotic system (ANATEC, Sweden) in conjunction with an automated inhouse module controlled by Labview software. Elemental analyses were carried out by Atlantic Microlab (Georgia, USA) and were within $\pm 0.4\%$ of theory. Flash chromatography used silica gel 60 (70–300 mesh). Radiochemical yields were determined with a dose calibrator (Capintec CRC-712M).

Mass spectrometry was conducted by the AIMS laboratory at the University of Toronto. Nuclear magnetic resonance spectra were recorded at 25°C on a Varian Mercury 300 MHz spectrometer with an autoswitchable H/F/C/P 5-mm probe with gradients. Chemical shifts were reported using tetramethylsilane (0.00 ppm) as an internal standard. All animal experiments were carried out under humane conditions, with approval from the Animal Care Committee at the Centre for Addiction and Mental Health and in accordance with the guidelines set forth by the Canadian Council on Animal Care.

4-Phenoxypyridin-3-amine (**1**) was prepared by modifications of a literature method [27]. A stirred mixture of 4-chloro-3-nitropyridine (5 g, 31.5 mmol), K_2CO_3 (7.7 g, 55.7 mmol) and phenol (4.0 g, 42.5 mmol) in DMF (50 ml) was heated to 70°C for 2 h then stirred overnight at ambient temperature. The reaction mixture was quenched with water (250 ml) and extracted with ethyl acetate (2×250 ml). Combined organic extracts were washed with saturated aqueous K_2CO_3 , water and brine; dried (Na_2SO_4) and filtered, and the solvent was removed to leave a red oil (6.23 g). The product was treated with MeOH (250 ml), $SnCl_2$ (37.4 g, 197 mmol) and 6 N HCl (50 ml) and refluxed for 16 h. After evaporation of MeOH, the residue was brought to pH>10 with 5 N NaOH and extracted with EtOAc (2×300 ml). Combined organic extracts were washed with water and brine, dried (Na_2SO_4) and filtered, and solvent was removed to leave a colourless oil which was dried under high vacuum (5.06 g, 86.3%). ^1H NMR($CDCl_3$) 8.15 (s, 1H), 7.89 (d, 1H, $J=5.4$ Hz), 7.06–7.43 (m, 5H), 6.57 (d, 1H, $J=5.4$ Hz), 3.89 (2H, br.s.).

4.1.1. *N*-(2-methoxybenzyl)-*n*-(4-phenoxypyridin-3-yl)acetamide (PBR28)

4-Phenoxypyridin-3-amine (330 mg, 1.77 mmol) and *o*-anisaldehyde (340 mg, 2.5 mmol) were stirred and heated to 100°C for 1 h. The mixture was cooled to 0°C in an ice bath, and MeOH (5 ml) was added, followed by $NaBH_4$ (0.2 g, 5.3 mmol) in portions over 20 min. The ice bath was removed, and the mixture was stirred at ambient temperature for a further 30 min. Two drops of acetic acid were then added, and stirring continued for a further 10 min. Volatiles were removed (rotary evaporator), and the residue was partitioned between EtOAc and saturated aqueous K_2CO_3 . The aqueous layer was extracted with EtOAc, and the combined organic extracts were washed with brine, dried (Na_2SO_4), filtered and evaporated to yield a pale yellow oil. The oil was taken up in anhydrous dichloromethane (DCM, 5 ml) and treated with 4-dimethylaminopyridine (5 mg), followed by 5 ml of 17% acetyl chloride in DCM (v/v). The reaction was

quenched by addition of aqueous K_2CO_3 (1M, 10 ml). The organic layer was removed and the aqueous layer extracted with DCM. The combined organic extracts were washed with brine, dried (K_2CO_3) and filtered, and solvent was removed to leave a viscous yellow oil. Flash chromatography with EtOAc/hexane/ Et_3N (90:10:1) to EtOAc/ Et_3N (98:2) gave PBR28 as a colourless oil, which solidified upon drying under vacuum (0.45 g, 73%). A portion was recrystallised from EtOAc/hexane to give a white solid: mp 90–91°C (lit. [26] 92.5–93°C). ^1H NMR($CDCl_3$) 8.27 (d, 1H, $J=5.7$ Hz), 8.20 (s, 1H), 7.14–7.44 (m, 5H), 6.84–6.93 (m, 2H), 6.74 (m, 2H), 6.55 (1H, d, $J=5.6$ Hz), 5.15 (d, 1H, $J=14.2$ Hz), 4.89 (d, 1H, $J=14.2$ Hz), 3.57 (s, 3H), 1.99 (s, 3H). ^{13}C NMR (acetone- d_6) 169.6, 160.8, 158.0, 153.9, 152.0, 150.8, 131.0, 130.6, 129.1, 129.0, 126.1, 125.5, 121.0, 120.5, 110.7, 110.6, 54.9, 45.5, 21.6. HRMS (M+1) $C_{21}H_{21}N_2O_3$ calc. 349.1546 measured 349.1546. Anal. ($C_{21}H_{20}N_2O_3$) C, H, N.

4.1.2. 2-((4-phenoxypyridin-3-ylamino)methyl)phenol (**2**)

4-Phenoxypyridin-3-amine (454 mg, 2.44 mmol) and *o*-salicylaldehyde (450 mg, 3.68 mmol) were stirred and heated to 90°C for 1 h. The mixture was cooled to 0°C in an ice bath, and MeOH (6 ml) was added, followed by $NaBH_4$ (0.25g, 6.6 mmol) in portions over 20 min. The ice bath was removed, and the mixture was stirred at ambient temperature for a further 30 min. Formic acid (0.2 ml) was then added and stirred for a further 15 min. Volatiles were removed (rotary evaporator) and the residue stirred with a mixture of EtOAc (20 ml) and saturated aqueous $NaHCO_3$ (20 ml). The resultant precipitate was collected by vacuum filtration, washed with a little EtOAc and dried in vacuo, yielding a white solid (0.55 g, 78%): mp 180–182°C, ^1H NMR (DMSO- d_6) 7.85 (s, 1H), 7.68 (d, 1H, $J=5.2$ Hz), 7.00–7.47 (m, 7H), 6.67–6.83 (m, 2H), 6.52 (d, 1H, $J=5.1$ Hz), 5.88 (t, 1H, $J=6.3$ Hz), 4.33 (d, 2H, $J=6.3$ Hz). HRMS $C_{18}H_{16}N_2O_2$ calc. 292.121178 measured 292.121260.

4.1.3. *N*-(2-hydroxybenzyl)-*n*-(4-phenoxypyridin-3-yl)acetamide (**3**)

The 2° amine, (**2**), (0.517g, 1.77 mmol) and 4-dimethylaminopyridine (5 mg) in DCM (dry, 5 ml) was stirred and treated with 5 ml of 17% acetyl chloride in DCM (v/v) dropwise. After 24 h, the DCM was evaporated off and sat. LiOH in MeOH (5 ml) added to the residue. After stirring for 10 min, water (10 ml) was added and MeOH removed on a rotary evaporator. The aqueous solution was extracted with EtOAc twice, and the combined extracts were washed with brine, dried (Na_2SO_4) and filtered, and solvent was removed to leave an oily solid. Trituration with hexane followed by recrystallisation from EtOAc/hexane gave a white solid (0.53g, 89.5%): mp 123–125°C. ^1H NMR ($CDCl_3$) 9.22 (br. s., 1H), 8.42 (d, 1H, $J=5.6$ Hz), 8.31 (s, 1H), 6.61–7.44 (m, 10H), 4.82 (s, 2H), 2.02 (s, 3H); HRMS (M+1) $C_{20}H_{19}N_2O_3$ calc. 335.1390; measured 335.1379. Anal. ($C_{20}H_{18}N_2O_3$) C, H, N.

4.1.4. *N*-(2-((*n*-4-phenoxy)pyridin-3-yl)acetimido)methyl phenoxyethyl 4-methylbenzenesulphonate (**4**)

An oven-dried flask was charged with NaH (0.27 g, 60%, 6.2 mmol) and stirred under N₂. A solution of the acetamide (**3**) (2.23 g, 6.67 mmol) in DMF (25 ml) was added and stirred at ambient temperature for 10 min. A solution of ethyleneglycol bistosylate (recrystallised from chloroform, 3.5 g, 9.45 mmol) in DMF (15 ml) was added in one portion. The reaction mixture was stirred under N₂ for 50 min, quenched with water (300 ml) and extracted twice with EtOAc. Combined extracts were washed thrice with water and brine, dried (MgSO₄) and filtered, and solvent was removed to leave a brown solid (2.6 g). Gradient flash chromatography [EtOAc:hexane (50:50) through EtOAc, then 1–5% MeOH in EtOAc] was performed twice to yield a waxy solid (1.77 g, 50%). A portion (0.89 g) was recrystallised from 95% aqueous ethanol to give white crystals (0.59 g): mp 106–108°C. ¹H NMR (CDCl₃) 8.25 (d, 1H, *J*=5.6 Hz), 8.16 (s, 1H), 7.78 (d, 1H, *J*=8.4 Hz), 6.86–7.48 (m, 11H), 6.63 (d, 1H, *J*=5.7 Hz), 6.54 (d, 1H, *J*=5.7 Hz), 5.04 (d, 1H, *J*=14.4 Hz), 4.84 (d, 1H, *J*=14.2 Hz), 3.91–4.22 (m, 4H), 2.43 (s, 3H), 1.98 (s, 3H); HRMS (*M*+1) C₂₉H₂₉N₂O₆S calc. 533.1740, measured 533.1762. Anal. (C₂₉H₂₈N₂O₆S) C, H, N.

4.1.5. *N*-(2-(2-fluoroethoxy)benzyl)-*n*-(4-phenoxy)pyridin-3-yl)acetamide

A stirred solution of the acetamide (**3**) (1.0 g, 3.0 mmol) in DMF (15 ml) was treated with tetrabutylammonium hydroxide (1 N in MeOH, 3 ml), followed by a solution of 2-fluoroethyl tosylate (0.90 g, 4.1 mmol) in DMF (2 ml) [28]. After stirring for 16 h at 40°C, water (100 ml) was added and the mixture extracted twice with EtOAc. The combined extracts were washed with saturated aqueous K₂CO₃ and brine, dried (Na₂SO₄) and filtered. Removal of solvent left a brown oil (1.03 g). Gradient flash chromatography [EtOAc:hexane (50:50) through EtOAc, then 1–5% MeOH in EtOAc] gave pure product as a viscous colourless oil (0.48 g, 42%). Treatment with ethereal HCl followed by recrystallisation from acetone gave white plates of the hydrochloride salt: mp 181–185°C. Free base ¹H NMR (CDCl₃) 8.21 (d, 1H, *J*=5.7 Hz), 8.135 (s, 1H), 6.8–7.5 (m, 7H), 6.67 (d, 1H, *J*=8.2 Hz), 6.51 (d, 1H, *J*=5.6 Hz), 5.16 (d, 2H, *J*=14.2 Hz), 4.82 (d, 2H, *J*=14.2 Hz), 4.32–4.65 (m, 2H), 3.80–4.08 (m, 2H), 1.92 (s, 3H); ¹³C NMR (CDCl₃) 170.8, 160.9, 156.6, 153.5, 150.8, 131.8, 130.5, 129.2, 128.9, 126.1, 125.5, 121.4, 120.9, 111.4, 110.8, 81.9 (d, *J*=174 Hz), 67.3 (d, *J*=20 Hz), 46.2, 22.5. HRMS (*M*+1) C₂₂H₂₂N₂O₆F calc. 381.1608 measured 381.1615. Anal. (C₂₂H₂₂N₂O₆FCI) C, H, N.

[¹¹C]-PBR 28. [¹¹C]-methylations were carried out inside an HPLC sample loop using our previously described method [29]. The normethyl precursor (**3**) (0.5 mg) was dissolved in DMF (77 μl), treated with tetrabutylammonium hydroxide in MeOH (3 μl, 1 N) and loaded onto an HPLC injection loop (2 ml) on a Valco HPLC injector valve in the LOAD position. [¹¹C]-CH₃I (produced as described previously [42]) was

swept into the HPLC loop coated with precursor solution by a stream of N₂ gas (8 ml/min) at ambient temperature. Radioactivity trapped on the loop is detected by a proximal radiation detector. When radioactivity peaked in the loop (3–4 min), the flow of N₂ was stopped, and after 2 min, the contents of the loop were quantitatively transferred onto the HPLC purification column by simply changing the position of the injection valve from the load to the inject position. The reaction mixture purified by semi-preparative HPLC; Phenomenex Luna(2)C18 10μ (250×10mm, 50% CH₃CN:50% H₂O+0.1 N NH₄HCO₂, 6 ml/min. The desired fraction (Rt 7.5 min) was collected in a flask and evaporated to dryness at 70°C under vacuum, and the residue was taken up in a mixture of 9 ml of sterile saline and 1 ml of ethanol. The saline solution of [¹¹C]-PBR28 was passed through a sterile 0.22 μm filter into a sterile, pyrogen-free bottle containing aqueous sodium bicarbonate (1 ml, 8.4%). An aliquot (100 μl) of the formulated solution was used to establish the chemical and radiochemical purity and specific activity of the final solution by analytical HPLC; Phenomenex ProdigyC18 10μ (250×4.5mm, 50% CH₃CN:50% H₂O+0.1 N NH₄HCO₂, 3 ml/min, Rt-product 3.0 min).

Further evidence for the identity of the radiolabelled product was obtained by coinjection of [¹¹C]-PBR28 with “cold” PBR 28 using a different conditions (solvents, pH, wavelength) with different analytical HPLC columns (Waters, Alltech, Phenomenex). Under all conditions, [¹¹C]-PBR28 cochromatographed with authentic PBR28.

[¹¹C]-PBR28-*d*3 was prepared in an identical fashion from (**3**) and [¹¹C]-CD₃I, which was prepared using LiAlD₄ to trap the cyclotron produced [¹¹C]-CO₂ [30] and used as describe above for [¹¹C]-CH₃I. No discernable difference in the production or characteristics of the isotopologues were observed.

4.2. Production of reactive [¹⁸F]-fluoride

Aqueous [¹⁸F]-fluoride was produced in a silver target by the ¹⁸O(p,n)¹⁸F reaction using 95% enriched [¹⁸O]H₂O (Rotem) employing a Scanditronix MC17 cyclotron. Target volume was 1 ml. At the end of bombardment, the target contents were transferred by helium pressure via 1/16" o.d. polytetrafluoroethylene tubing through a small ion exchange column (Dionex 1×8 200–400 mesh, bicarbonate form, 10–15 mg), trapping the [¹⁸F]-fluoride and enabling collection of the enriched [¹⁸O]H₂O for recycling [43]. The [¹⁸F]-fluoride was removed from the resin by back-flushing with 1 ml of a 96:4 (by volume) acetonitrile:water mixture containing 14.4 mg of 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (Kryptofix 222) and 3 mg of K₂CO₃ [31] into an open Vacutainer serum tube (7 ml). The contents of the tube were then assayed in a dose-calibrator and dried by heating at 90°C under a stream of nitrogen (100 ml/min) for 5–6 min.

4.2.1. Radiosynthesis of [¹⁸F]-FEPPA

To the dried [¹⁸F]-fluoride was added a solution of (**4**) (5 mg) in dry acetonitrile (0.7 ml), and the solution was

stirred for 10 min at 90°C. The reaction was quenched by addition of 30/70 MeOH/H₂O+0.5% formic acid (3 ml) and purified directly by HPLC (Phenomenex ProdigyC18 10 μ , 250 $\times$ 10 mm; 50:50 MeOH:H₂O+0.5% formic acid; 6 ml/min). The desired fraction (Rt_{FEPPA} 22 min) was collected and evaporated to dryness under vacuum at 70°C, and the residue taken up in 10 ml of sterile saline and 1 ml of USP ethanol. The ethanolic saline solution of [¹⁸F]FEPPA was passed through a sterile 0.22- μ m filter into a sterile, pyrogen-free bottle containing aqueous sodium bicarbonate (1 ml, 8.4%). Aliquots of the formulated solution was used to establish the chemical and radiochemical purity and specific activity of the final solution by analytical HPLC; Phenomenex Prodigy C18 10 μ (250 $\times$ 4.5 mm, 50% CH₃CN 50% H₂O+0.1 N ammonium formate 3 ml/min).

4.2.2. Metabolite studies

Rats (male, Sprague–Dawley, 300–400g) were injected with 50–100 MBq (for [¹¹C]-PBR28) or 10–15 MBq (for [¹⁸F]-FEPPA) (at time of first injection) of high specific activity radiotracer (1–3 nmol) in 0.3 ml of 10% ethanol in buffered saline via the tail vein, vasodilated in a warm water bath. Rats were killed at various time points post injection by decapitation, blood collected from the trunk in a heparinised tube and the whole brain surgically removed from the skull. Control animals, which received no radiotracer, were also killed; blood was collected, and the brain was excised and stored on ice. About 1 MBq of radiotracer in 10 μ l of saline was added to the control brains and control blood samples only. Brains were homogenised (Polytron, setting 7) in 5 ml of ice-cold 80% aqueous ethanol and centrifuged (17,000 rpm, 10 min). Pellets were resuspended in 1 ml of ice-cold 80% aqueous ethanol and recentrifuged. Aliquots of the supernatants and the pellets were counted for radioactivity; the supernatants were then diluted 4:1 (v/v) with water for HPLC analysis. Blood was centrifuged to separate the plasma, which was treated with 10% by volume of acetic acid then used directly for HPLC analysis. HPLC analysis of plasma and brain extracts were performed by minor modifications of the method described by Hilton et al. [44]. Briefly, samples were loaded onto a 5-ml HPLC injector loop (Valco, Texas, USA) and injected onto a small capture column (4.6 $\times$ 20 mm) packed in house with OASIS HLB 30 μ m (Waters, NJ). The capture column was eluted with 1% aqueous acetonitrile (2 ml/min) for 4 min then back-flushed (50% acetonitrile/50% H₂O + 0.1 N NH₄HCO₂, pH 4,) onto a Phenomenex 10 μ Luna C18 column (250 $\times$ 4.6 mm). Both column effluents were monitored through a flow detector (Bioscan Flow-Count) operated in coincidence mode. In some experiments, after elution of the parent radiotracer, the HPLC eluent was changed to 100% EtOH to monitor for strongly retained (lipophilic) metabolites. All radioactivity data were corrected for physical decay and integrated. Hold-up of radioactivity in the HPLC system was less than 2% of applied radioactivity, while the centrifuged brain pellet had less than 5%.

4.2.3. Log *P* measurements

The partition coefficients of [¹¹C]-PBR18 and [¹⁸F]-FEPPA, between 1-octanol and 0.02 M phosphate buffer at pH 7.4 were determined by a previously described method [32], replicated six times.

4.2.4. Binding assays

Mitochondrial tissue fractions from rat cortex were employed. Displacement assays were run in duplicate in compounds covering the range from 1 μ M–6 pM (12 serial 1/3 dilutions), using [³H]-PK11195 as the radiotracer.

4.2.5. Biodistribution in rats

Rats (male, Sprague–Dawley, 250–290 g) were kept on a reversed 12-h light/12-h dark cycle and allowed food and water ad libitum. A previously described method [45] was used to determine the regional brain uptake of radioactivity. Briefly, rats, in a restraining box, received 25–30 MBq (for [¹¹C]-PBR28) or 2–3 MBq (for [¹⁸F]-FEPPA) (at time of first injection) of high specific activity radiotracer (0.5–2 nmol) in 0.3 ml of 10% ethanol in buffered saline via the tail vein, vasodilated in a warm water bath. Rats were killed by decapitation at various time intervals after radiotracer administration, the brain removed and stored on ice. Brain regions were excised, blotted and weighed, while blood was collected (from the trunk). Radioactivity in tissues was assayed in an automated gamma counter, back corrected to time of injection, using diluted aliquots of the initial injected dose as standards.

4.2.6. Pharmacological challenge studies in rats

Groups of rats (*n*=6) received a coinjection of a solution (1 ml/kg body weight in 1% Tween 80 in saline iv) of the challenge drug and the radiotracer. Control animals (a control group was run each time) received only radiotracer in 1% Tween 80 in saline iv.

Acknowledgments

The authors would like to thank Winston Stableford, Min Wong, Alvina Ng and Ann-Noël Samaha for their technical assistance. We also thank Mr. Clavo, Dr. Dominguez and Dr. Bennacef of GlaxoSmithKline for performing the binding assays.

References

- [1] Papadopoulos V, Baraldi M, Guilarte TR, Knudsen TB, Lacapere JJ, Lindemann P, et al. Translocator protein (18kDa): new nomenclature for the peripheral-type benzodiazepine receptor based on its structure and molecular function. *Trends Pharmacol Sci* 2006;27:402–9.
- [2] Venneti S, Lopresti BJ, Wiley CA. The peripheral benzodiazepine receptor (translocator protein 18kDa) in microglia: from pathology to imaging. *Prog Neurobiol* 2006;80:308–22.
- [3] Pike VW, Halldin C, Crouzel C, Barré L, Nutt DJ, Osman S, et al. Radioligands for PET studies of central benzodiazepine receptors and PK (peripheral benzodiazepine) binding sites — current status. *Nucl Med Biol* 1993;20:503–25.

- [4] Junck L, Olson JM, Ciliax BJ, Koeppe RA, Watkins GL, Jewett DM, et al. PET imaging of human gliomas with ligands for the peripheral benzodiazepine binding site. *Ann Neurol* 1989;26:752–8.
- [5] Dubois A, Benavides J, Peny B, Duverger D, Fage D, Gotti B, et al. Imaging of primary and remote ischaemic and excitotoxic brain lesions. An autoradiographic study of peripheral type benzodiazepine binding sites in the rat and cat. *Brain Res* 1988;445:77–90.
- [6] Gavish M, Bachman I, Shoukrun R, Katz Y, Veenman L, Weisinger G, et al. Enigma of the peripheral benzodiazepine receptor. *Pharmacol Rev* 1999;51:629–50.
- [7] Gerhard A, Pavese N, Hotton G, Turkheimer F, Es M, Hammers A, et al. In vivo imaging of microglial activation with [¹¹C](R)-PK11195 PET in idiopathic Parkinson's disease. *Neurobiol Dis* 2006;21:404–12.
- [8] Cagnin A, Gerhard A, Banati RB. In vivo imaging of neuroinflammation. *Eur Neuropsychopharmacol* 2002;12:581–6.
- [9] Hashimoto K, Inoue O, Suzuki K, Yamasaki T, Kojima M. Synthesis and evaluation of 11C-PK 11195 for in vivo study of peripheral-type benzodiazepine receptors using positron emission tomography. *Ann Nucl Med* 1989;3:63–71.
- [10] Camsonne R, Crouzel C, Comar D, Mazière M, Prenant C, Sastre J, et al. Synthesis of *N*-(¹¹C)methyl, *N*-(methyl-1-propyl), (chloro-2-phenyl)-1-isoquinoline carboxamide-3 (PK-11195): a new ligand for peripheral benzodiazepine receptors. *J Labelled Comp Radiopharm* 1984;21:985–91.
- [11] Benavides J, Quarteronet D, Imbault F, Malgouris C, Uzan A, Renault C, et al. Labelling of "peripheral-type" benzodiazepine binding sites in the rat brain by using [³H]PK 11195, an isoquinoline carboxamide derivative: kinetic studies and autoradiographic localization. *J Neurochem* 1983;41:1744–50.
- [12] James ML, Selleri S, Kassiou M. Development of ligands for the peripheral benzodiazepine receptor. *Curr Med Chem* 2006;13:1991–2001.
- [13] Okubo T, Yoshikawa R, Chaki S, Okuyama S, Nakazato A. Design, synthesis, and structure-activity relationships of novel tetracyclic compounds as peripheral benzodiazepine receptor ligands. *Bioorg Med Chem* 2004;12:3569–80.
- [14] James ML, Fulton RR, Henderson DJ, Eberl S, Meikle SR, Thomson S, et al. Synthesis and in vivo evaluation of a novel peripheral benzodiazepine receptor PET radioligand. *Bioorg Med Chem* 2005;13:6188–94.
- [15] Boutin H, Chauveau F, Thominiaux C, Gregoire MC, James ML, Trebossen R, et al. 11C-DPA-713: a novel peripheral benzodiazepine receptor PET ligand for in vivo imaging of neuroinflammation. *J Nucl Med* 2007;48:573–81.
- [16] Chaki S, Funakoshi T, Yoshikawa R, Okuyama S, Okubo T, Nakazato A, et al. Binding characteristics of [³H]DAA1106, a novel and selective ligand for peripheral benzodiazepine receptors. *Eur J Pharmacol* 1999;371:197–204.
- [17] Zhang MR, Kida T, Noguchi J, Furutsuka K, Maeda J, Suhara T, et al. [¹¹C]DAA1106: radiosynthesis and in vivo binding to peripheral benzodiazepine receptors in mouse brain. *Nucl Med Biol* 2003;30:513–9.
- [18] Maeda J, Suhara T, Zhang MR, Okauchi T, Yasuno F, Ikoma Y, et al. Novel peripheral benzodiazepine receptor ligand [¹¹C]DAA1106 for PET: an imaging tool for glial cells in the brain. *Synapse* 2004;52:283–91.
- [19] Zhang MR, Maeda J, Furutsuka K, Yoshida Y, Ogawa M, Suhara T, et al. [¹⁸F]FMDAA1106 and [¹⁸F]FEDAA1106: two positron-emitter labeled ligands for peripheral benzodiazepine receptor (PBR). *Bioorg Med Chem Lett* 2003;13:201–4.
- [20] Briard E, Hong J, Musachio JL, Zoghbi SS, Fujita M, Imaizumi M, et al. Synthesis and evaluation of two candidate 11C-labeled radioligands for brain peripheral benzodiazepine receptors. *J Label Compd Radiopharm* 2005;48:S71.
- [21] Imaizumi M, Kim HJ, Zoghbi SS, Briard E, Hong J, Musachio JL, et al. PET imaging with [¹¹C]PBR28 can localize and quantify upregulated peripheral benzodiazepine receptors associated with cerebral ischemia in rat. *Neurosci Lett* 2007;411:200–5.
- [22] Fujimura Y, Ikoma Y, Yasuno F, Suhara T, Ota M, Matsumoto R, et al. Quantitative Analyses of 18F-FEDAA1106 binding to peripheral benzodiazepine receptors in living human brain. *J Nucl Med* 2006;47:43–50.
- [23] Ikoma Y, Yasuno F, Ito H, Suhara T, Ota M, Toyama H, et al. Quantitative analysis for estimating binding potential of the peripheral benzodiazepine receptor with [(11)C]DAA1106. *J Cereb Blood Flow Metab* 2007;27:173–84.
- [24] Imaizumi M, Briard E, Zoghbi S, Hong J, Musachio J, Gourley J, et al. Quantification of peripheral benzodiazepine receptors using a new aryloxyanilide based ligand [¹¹C]PBR28 in monkey and human. *J Nucl Med* 2007;48:242P [Meeting Abstracts].
- [25] Imaizumi M, Briard E, Zoghbi S, Hong J, Shah J, Musachio J, et al. Kinetic and metabolic evaluation of new PET ligands for peripheral benzodiazepine receptors. *J Nucl Med* 2006;47:499P [Meeting Abstracts].
- [26] Nakazato A, Okubo T, Nakamura T, Chagi S, Tomisawa K, Nagamine M, et al. Aryloxy nitrogen-containing heteroarylamines derivative, their use and MDR ligands containing them. Japanese Patent 001476; 2000, p. 001476 (patent).
- [27] Mann FG. Xanthenes and thioxanthenes. Part VI. The preparation and properties of 9-thia-3-aza-anthrone. *J Chem Soc* 1955:2755–63.
- [28] Wilson AA, DaSilva JN, Houle S. [¹⁸F]Fluoroalkyl analogues of the potent 5-HT_{1A} antagonist WAY 100635: radiosyntheses and in vivo evaluation. *Nucl Med Biol* 1996;23:487–90.
- [29] Wilson AA, Garcia A, Jin L, Houle S. Radiotracer synthesis from [¹¹C]-iodomethane: a remarkably simple captive solvent method. *Nucl Med Biol* 2000;27:529–32.
- [30] Coumbarides G, Eames J, Weerasooriya N. A practical laboratory route to the synthesis of trideuteriomethyl-[¹³C] iodide. *J Label Compd Radiopharm* 2003;46:291–6.
- [31] Gomzima NA, Vasil'ev DA, Krasikova RN. Optimization of Automated Synthesis of 2-[¹⁸F]Fluoro-2-deoxy-d-glucose involving base hydrolysis. *Radiokhimiya* 2002;44:366–72.
- [32] Wilson AA, Jin L, Garcia A, DaSilva JN, Houle S. An admonition when measuring the lipophilicity of radiotracers using counting techniques. *Appl Rad Isot* 2001;54:203–8.
- [33] Leo AJ. Calculating log P_{oct} from structures. *Chem Rev* 1993;93:1281–306.
- [34] Ding YS, Fowler JS, Wolf AP. Rapid, regiospecific syntheses of deuterium substituted 6- [¹⁸F]fluorodopamine (alpha, alpha-D2 beta, beta-D2 and alpha, alpha, beta, beta-D4) for mechanistic studies with positron emission tomography. *J Label Compound Radiopharm* 1993;33:645–54.
- [35] Fowler JS, Wang GJ, Logan J, Xie S, Volkow ND, Macgregor RR, et al. Selective reduction of radiotracer trapping by deuterium substitution: Comparison of carbon-11-deprenyl and carbon-11-deprenyl-D2 for MAO B mapping. *J Nucl Med* 1995;36:1255–62.
- [36] Zhang MR, Maeda J, Ito T, Okauchi T, Ogawa M, Noguchi J, et al. Synthesis and evaluation of *N*-(5-fluoro-2-phenoxyphenyl)-*N*-(2-[(18)F]fluoromethoxy-d(2)-5-methoxybenzyl)acetamide: a deuterium-substituted radioligand for peripheral benzodiazepine receptor. *Bioorg Med Chem* 2005;13:1811–8.
- [37] Venneti S, Lopresti BJ, Wang G, Slagel SL, Mason NS, Mathis CA, et al. A comparison of the high-affinity peripheral benzodiazepine receptor ligands DAA1106 and (R)-PK11195 in rat models of neuroinflammation: implications for PET imaging of microglial activation. *J Neurochem* 2007.
- [38] Waterhouse RN. Determination of lipophilicity and its use as a predictor of blood-brain barrier penetration of molecular imaging agents. *Mol Imaging Biol* 2003;5:376–89.
- [39] Suhara T, Sudo Y, Yoshida K, Okubo Y, Fukuda H, Obata T, et al. Lung as reservoir for antidepressants in pharmacokinetic drug interactions. *Lancet* 1998;351:332–5.

- [40] Imaizumi M, Briard E, Zoghbi SS, Gourley JP, Hong J, Musachio JL, et al. Kinetic evaluation in nonhuman primates of two new PET ligands for peripheral benzodiazepine receptors in brain. *Synapse* 2007;61: 595–605.
- [41] Rigorous kinetic modeling is required for a proper assessment of the displacement of [^{18}F]-FEPPA and [^{11}C]-PRB28 by the cold FEPPA or PRB28, however the following argument can be made to support specific displacement of these two radioligands from the brain PBRs. Although a stable equilibrium state between brain and plasma was not achieved during our studies, the region to plasma ratios do remain proportional to the distribution volumes of the two compounds. With increasing plasma levels, the brain to blood ratios should not be affected under tracer conditions. In our experiments, this ratio actually decreased with increased blocking doses while the plasma levels increased due to blocking of the peripheral PBR binding sites. One can infer from these lower ratios that the specific brain binding to PBRs is significantly reduced in the blocked conditions.
- [42] Wilson AA, DaSilva JN, Houle S. Facile radiolabelling and purification of 2 β -[O- ^{11}C]-carbomethoxy-3 β -aryltropanes: radiotracers for the dopamine transporter. *J Labelled Compd Radiopharm* 1994;34:759–65.
- [43] Schleyer DJ, Bastos MA, Alexoff D, Wolf AP. Separation of [^{18}F] Fluoride from [^{18}O]Water using anion exchange resin. *Appl Radiat Isot Part A* 1990;41:531–3.
- [44] Hilton J, Yokoi F, Dannals RF, Ravert HT, Szabo Z, Wong DF. Column-switching HPLC for the analysis of plasma in PET imaging studies. *Nucl Med Biol* 2000;27:627–30.
- [45] Wilson AA, DaSilva JN, Houle S. In vivo evaluation of [^{11}C] and [^{18}F]-labelled cocaine analogues as potential dopamine transporter ligands for positron emission tomography. *Nucl Med Biol* 1996;23:141–6.