Published in final edited form as:

Neuroimage. 2007 February 15; 34(4): 1352–1362.

Quantification of $\alpha 4\beta 2^*$ nicotinic receptors in the rat brain with microPET® and 2-[^{18}F]F-A-85380

D. Bruce Vaupel, Elliot A. Stein, and Alexey G. Mukhin

Neuroimaging Research Branch, National Institute on Drug Abuse, Intramural Research Program, National Institutes of Health, Department of Health and Human Services, Baltimore, MD 21224.

Abstract

The radioligand 2-[^{18}F]F-A-85380 has been used for PET studies of the $\alpha 4\beta 2^*$ subtype of nicotinic acetylcholine receptors (nAChRs) in the living brain of humans and nonhuman primates. In order to extend the capacity of microPET to quantify neuroreceptors in rat brain, we carried out studies of 2-[^{18}F]F-A-85380 to measure the apparent binding potential BP^* in individual rats, which were studied repeatedly over several months. Using a bolus-plus-infusion paradigm, 2-[^{18}F]F-A-85380 (specific activity 20 - 1300 GBq/ μmol) was administered intravenously over 8 to 9 h with K_{bol} values of 350 to 440 min and a mean infusion rate of 0.03 ± 0.01 nmol/kg/h. Studies included a 2-h nicotine infusion initiated 2 h before the end of scanning to displace specifically bound radioactivity. Steady state binding in brain was obtained within 5 h as defined by the occurrence of constant radioactivity concentrations in brain regions and constant, free arterial plasma levels of nonmetabolized radioligand. BP^* averages ($\pm$ SEM) for thalamus, forebrain, and cerebellum were 5.9 ± 0.7 , 2.6 ± 0.4 , and 1.0 ± 0.1 , respectively, which are consistent with the $\alpha 4\beta 2^*$ nAChR distribution in rat brain measured *in vitro*. Studies of receptor occupancy determined the ED_{50} to be 0.29 nmol/kg/h. The demonstration that $\alpha 4\beta 2^*$ nAChRs are quantifiable in the rat brain using PET measurements, coupled with the ability to conduct longitudinal studies over several months in the same rats, suggests potential applications to studies of chronic nicotine use, its treatment, and abnormal functioning of $\alpha 4\beta 2^*$ receptors in a rat model.

Introduction

Actions of the endogenous neurotransmitter acetylcholine are mediated by two classes of receptors with distinct pharmacologies. Whereas nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels, which become permeable to calcium ions when activated by nicotinic agonists, muscarinic acetylcholine receptors belong to the class of G-protein coupled receptors. The nAChRs are heteropentamers, composed of diverse possible combinations of subunits. However, the main types occurring in the mammalian brain are the $\alpha 4\beta 2^*$ and $\alpha 7$ subtypes (Lindstrom et al., 1995; Holladay et al., 1997), where the asterisk denotes that one or more additional subunit types may be part of the pentameric complex. 2-[^{18}F]F-A-85380 is a radioligand specifically developed to image the $\alpha 4\beta 2^*$ nAChR subtype using positron emission tomography (PET) (Koren et al., 1998; Horti et al., 1998; Valette et al., 1999) and is

Correspondence to: Dr. D. Bruce Vaupel, NIDA IRP, Neuroimaging Research Branch, 5500 Nathan Shock Drive, Baltimore, MD, 21224, Voice: 410-550-1440 ext 330, Fax: 410-550-1441, email: bvaupel@intra.nida.nih.gov

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

A preliminary report of this research (NeuroImage 31, 2006, T103) was made at The Sixth International Symposium on Neuroreceptor Mapping, July 6-8, 2006, Copenhagen, Denmark.

characterized by high receptor selectivity for the $\alpha 4\beta 2^*$ receptor subtype relative to the $\alpha 7$, $\alpha 3\beta 4^*$, and muscle subtype nAChRs (Pavlova et al., 2000).

High selectivity for $\alpha 4\beta 2^*$ receptors likely contributes to the wide margins of safety that characterize $2[^{18}\text{F}]\text{F-A-85380}$ -like agonist compounds (Vaupel, 2003;2005). Consequently, the clinical use of $2[^{18}\text{F}]\text{F-A-85380}$ for PET imaging is continuing, whereas the use of PET radioligands developed from analogues of the high affinity, natural toxin epibatidine, such as NFEP (Molina et al., 1997;Ding et al., 1999), did not advance to human trials because the inherent cardiovascular and convulsant toxicities of the parent agonist compound had not been adequately overcome. At present, published human imaging studies (see below) using $2[^{18}\text{F}]\text{F-A-85380}$ have reported no adverse effects.

$2[^{18}\text{F}]\text{F-A-85380}$ has been successfully used with PET to image $\alpha 4\beta 2^*$ nAChRs in both humans (Bottlaender et al., 2003;Kimes et al., 2003;Gallezot et al., 2005;Mitkovski et al., 2005;Brody et al., 2006) and nonhuman primates (Chefer et al., 2003a;Valette et al., 2002). Developing imaging strategies to use this radioligand in the rat is of interest because of the widespread use of this species as an animal model to investigate the effects of nicotine. Behavioral paradigms involving repeated nicotine administration are frequently used in drug abuse research. These paradigms include motivational reward assessment (conditioned place preference), reinforcement (self-administration), dependence, precipitated withdrawal, and reinstatement of nicotine self-administration (see review by Le Foll and Goldberg, 2006). Other substantive research of nAChR subtypes in the rat includes evaluations of molecular characteristics, physiological properties, pharmacological selectivity, and distribution patterns of nAChRs within the brain. From an imaging perspective, PET scanners developed to image small animals offer the advantage of having better spatial resolution (1.8 mm) than full-size PET scanners (c.a. 4 mm), but have a lower sensitivity compared with full size scanners. The current projects seeks to extend the utility of microPET to the quantification of $\alpha 4\beta 2^*$ nAChRs in the brain of living rats using the radioligand $2[^{18}\text{F}]\text{F-A-85380}$.

To this end, our objective was to develop a simplified method using the apparent binding potential BP^* for the noninvasive quantification of $\alpha 4\beta 2^*$ nAChRs in the rat brain in the absence of a suitable reference region (Ichise et al., 2001). Experimental constraints that were imposed included a single experimental session, elimination of arterial blood sampling and kinetic modeling, and having the capability to conduct repeated studies over time. We successfully fulfilled the objectives of the experiments using a bolus-plus-infusion (B+I) administration paradigm of $2[^{18}\text{F}]\text{F-A-85380}$ that incorporated a nicotine displacement component.

Materials and methods

Animal preparation and experimental procedures

Experiments were conducted under a protocol approved by the Animal Care and Use Committee of the National Institute on Drug Abuse Intramural Research Program. Sprague-Dawley rats (Charles River Laboratories, Inc, Wilmington, MA) weighing 300 g at the time of their arrival were singly housed in an animal housing facility and were maintained on a 12-h light/12-h dark cycle (lights on at 7:00 am). Food and water were available *ad libitum*. During the periods of experimentation animal weights ranged between 470 and 700 g.

Anesthesia was induced by exposing rats to 4% to 5% isoflurane in oxygen. The eyes were coated with a protective ointment, and the local anesthetic Marcaine was placed in each ear before the rat was placed in a head holder. Once the head was immobilized in a custom-built head holder, which is located within an enclosure that surrounds the entire head to provide a closed system, the anesthetic machine was connected to the system. At this time the isoflurane

was reduced to 1.6 to 2.0% for the duration of the studies. Body temperature was maintained by a water-filled heating pad and monitored rectally. Heart rate and pO_2 were measured with a Vet/Ox G2 digital monitor (Heska Corp., Loveland, CO), and respiratory rate was visually counted. The two lateral tail veins were acutely catheterized for administration of radioligand alone or radioligand followed a displacing dose of nicotine. Subcutaneous 5% dextrose (ca. 6 ml) was administered in divided doses, typically at the 3-h and 6-h time points of the 9-h experiments. At the completion of the studies, rats were allowed to recover from anesthesia and were used in subsequent imaging studies. The minimum interval between experiments was 14 days, although a 3- to 4-week interval was more typical.

Radiosynthesis of 2-[^{18}F]F-A-85380

2-[^{18}F]F-A-85380 was prepared on-site using the procedure reported by Horti *et al.* (1998). The radioligand was formulated as a sterile apyrogenic saline solution. Total synthesis time ranged from 135 to 150 min. Radiochemical purity exceeded 99%.

Acquisition of PET images

PET images were acquired with a microPET Primate 4 tomograph (Concorde Microsystems Inc., Knoxville, TN). The detector geometry is defined by 4 rings, each having 42 detectors, together providing a cylindrical field of view measuring 22 cm in diameter with a 7.8-cm axial length. Lower and upper level energy windows were set at 350 and 750 keV, respectively, and a 6-nsec timing window for coincidence detection was used. Using the full 3D mode, all data were acquired in a list mode format that were subsequently sorted into 2D sinograms by using Fourier rebinning (Defrise *et al.*, 1997), which yielded 63 sinogram planes with a 1.2 mm axial sampling distance. Sinograms were reconstructed by standard 2D filtered back projection using a Ramp filter, a Nyquist cutoff of 0.5, and a zoom of 5. Corrections for dead time, random scattering, and attenuation were performed for all scans. See Tai *et al.* (2001) for additional information regarding microPET performance.

Experimental design: B+I with radioligand displacement

Using the B+I paradigm, 2-[^{18}F]F-A-85380 (overall specific activity 20 to 1300 GBq/ μ mol; 500 to 34,000 Ci/mmol) was continuously administered intravenously over 8 to 9 h. For studies limited to estimating BP* using high specific activity, the range of specific activities was 200 to 1300 GBq/ μ mol. In order to perform the receptor occupancy study, the specific activity of 2-[^{18}F]F-A-85380 was intentionally reduced with cold compound to achieve the desired low specific activities (20 to 200 GBq/ μ mol). The emission recording consisted of a series of 45 to 47 frames increasing in duration as follows: 5×120 s, 8×300 s, 10×600 s, and either $22 \times$ or 26×900 s. One minute before starting the emission scan, the priming volume to fill the catheter dead space was delivered at 24 ml/h for 1 min. At 0 min, the emission scan and bolus administration were started. The initial selection of $K_{bol} = 400$ min was based on calculations using the tissue radioactivity curves acquired from three preliminary simple bolus experiments. The bolus dose was infused over 5 min (24 ml/h) after which the infusion rate was reduced to 0.343 ml/h to deliver the remaining dose throughout the experiment. The range of K_{bol} values evaluated in 17 B+I experiments was 350 to 440 min. Rates of 2-[^{18}F]F-A-85380 infusion averaged 0.032 ± 0.013 ($\pm$ SD) nmol/kg/h.

The B+I studies also incorporated a pharmacological challenge component using unlabeled nicotine to displace specifically bound 2-[^{18}F]F-A-85380, described as a stimulus-induced change in specific binding of the radiotracer by Carson *et al.* (1997). The 2-h nicotine infusion was initiated 2 h before the end of the emission scan. It consisted of a 15-min fast infusion segment (0.4 mg/kg/h) followed by a 105-min slow infusion (0.1 mg/kg/h). Rates refer to the free base form of nicotine (Sigma-Aldrich, St. Louis, MO, USA).

Image analysis

Image data were acquired with microPET software (ASIPro, v 6.0.3.0 and microPET Manager, v 2.2.2.0, Concorde Microsystems Inc. Knoxville, TN, USA). Imaged data were analyzed using PMOD software v.2.7 (PMOD Technologies Ltd., Zurich, Switzerland). A standard set of regions of interest (ROIs) was established for determining time-activity curves (TACs) and BP*. Manually drawn ROIs for the thalamus, forebrain, and cerebellum initially were placed on coronal PET images of total radioactivity, which were averaged from 5 to 7 h (i.e., 6-h average value), during which time steady state conditions were present. Placement of these ROIs was guided by reference to a stereotactic atlas (Paxinos et al., 2000) and a set of magnetic resonance imaging (MRI) images of a Sprague-Dawley rat brain acquired using a 9.4 T Bruker BioSpec System, model 94/30USR (Bruker BioSpec MRI, Inc, Billerica, MA, USA). When these ROIs were placed on appropriate planes of the coronal images of nondisplaceable radioactivity for the last 3 frames (75 to 120 min of the nicotine infusion), there was an apparent spillover effect on forebrain and cerebellum ROIs resulting from ^{18}F accumulation in the skull bone as assessed by activity profiles. To reduce the effect of spillover, these ROIs were reduced in size by approximately 25%. These adjusted ROIs became the final set of ROIs that were placed on all dynamic scans for all studies to determine TACs.

Estimation of BP*

BP* values for 2- ^{18}F]F-A-85380 were determined from tissue radioactivity concentrations obtained from ROI analyses before and after the nicotine challenge using equation 4, which is derived below. The concentration of radioactivity due to the specific binding (C_{SB}) of 2- ^{18}F] F-A-85380 was the difference between the average 6-h concentration of total radioactivity (C_{Tot}) and the concentration of nondisplaceable radioactivity (C_{ND}) averaged over 8.4 to 9 h, which corresponds to the 98th ND minute of the nicotine infusion (elapsed study time of 8 h 38 min).

The binding potential, BP*, is defined by the ratio of the volume of distribution (VD) of the specifically bound compartment, $VD_{\text{SB}} = \frac{C_{\text{SB}}}{C_{\text{P}}}$, to the volume of distribution of the

nondisplaceable compartment, $VD_{\text{ND}} = \frac{C_{\text{ND}}}{C_{\text{P}}}$ where C_{SB} and C_{ND} are the specifically bound and nondisplaceable bound concentrations of radioactivity in the ROIs, respectively, and C_{P} is the plasma concentration radioactivity of free radioligand.

As defined above

$$BP^* = \frac{VD_{\text{SB}}}{VD_{\text{ND}}} \quad (1)$$

If F represents the free concentration of radioligand in tissue at equilibrium, F equals C_{P} . Making the corresponding substitutions of F for C_{P} in the formulas for VD_{ND} and VD_{SB} shown above results in equation (1) becoming

$$BP^* = \frac{\frac{C_{\text{SB}}}{F}}{\frac{C_{\text{ND}}}{F}} \quad (2)$$

Since the concentration of specifically bound radioactivity is defined as,

$$C_{\text{SB}} = C_{\text{Tot}} - C_{\text{ND}} \quad (3)$$

then BP* can be expressed as a function of the total and nondisplaceable radioactivity concentrations as follows,

$$BP^* = \frac{\frac{C_{Tot} - C_{ND}}{F}}{\frac{C_{ND}}{F}} = \frac{C_{Tot}}{C_{ND}} - 1 \quad (4)$$

In some experiments when arterial blood was available to determine C_p, V_D values were calculated as described above to validate the equilibrium conditions.

Mass dose and receptor occupancy

A total of 17 experiments, obtained from four rats tested with 3 to 5 different mass infusion rates, were used to determine the extent to which mass effects can influence the magnitude of BP* values, particularly its underestimation. These studies of receptor occupancy used larger mass doses of radioligand infusion (0.017 - 1.163 nmol/kg/h). The true BP* and ED₅₀ were determined from the data sets for individual rats. Next, the observed BP* for each experiment was expressed as a percent of the true BP* and plotted against the mass dose infusion rate for all experiments across all rats. From this curve, we determined the ED₅₀ for the mass dose infusion rate, which is defined as the rate that reduces the observed BP* value by one half.

Arterial blood sampling

Serial caudal arterial blood samples were acquired using individual arterial punctures from eight studies, and nonmetabolized concentrations of 2-[¹⁸F]F-A-85380 were determined using solid phase extraction (SPE) with Clean Screen™ extraction columns and four successive steps of washing with 0.5M NaHCO₃, H₂O, 1% acetic acid, and ethanol (Shumway et al., 2006). In practice it was difficult to acquire arterial samples at precise times, consequently reproducibility between experiments was estimated as follows. A biexponential function was fitted using Microsoft Excel Solver™ (Microsoft Corp., Redmond, WA, USA) to the measured plasma radioactivity concentrations of nonmetabolized 2-[¹⁸F]F-A-85380 for each study (5 to 12 samples collected over a period of 2 to 9 h). Next, plasma concentration values were calculated from each fitted curve for specific time intervals. These concentration values were averaged over the eight studies, the standard deviations were obtained, and a biexponential function fitted to these averages.

Results

Establishment of steady state conditions using the B+I paradigm

Development of the B+I paradigm was based on an initial K_{bol} value of 400 min, which was determined from three single bolus studies. In Fig. 1, the TACs for the bolus administration of 2-[¹⁸F]F-A-85380 are illustrated. Radioactivity peaked first in the cerebellum, followed in order by forebrain, cortex, and thalamus. Based on the percentages of the injected dose, the uptake of radioactivity in different regions of the rat brain was greater than that reported for comparable regions in the rhesus monkey (Chefer et al., 2003a) and baboon (Valette et al., 2002).

Using a K_{bol} of 400 min for the B+I administration of 2-[¹⁸F]F-A-85380, steady state concentrations of radioactivity in the brain developed by 5 h (Fig. 2). The infusion of nicotine was initiated at 7 h, and it effectively displaced the specifically bound radioligand with an average T_{1/2} for the washout of radioactivity from tissue of 17 min (Table 3). New steady state levels of activity corresponding to nondisplaceable radioactivity were nearly achieved during the last 45 min of the infusion (Fig. 2). This time corresponds to the last three emission scans.

Free arterial plasma concentrations of 2-[¹⁸F]F-A-85380

Samples of arterial blood were taken from the caudal tail artery in selected studies to demonstrate steady state conditions and to determine VD values (Table 3). Plasma concentrations of nonmetabolized 2-[¹⁸F]F-A-85380 acquired from arterial blood samples stabilized by 5 h and remained constant to the end of the 9-h study (Fig. 2, Fig. 3A, and 3B). To verify the development of steady state conditions, the radioactivity in the free fraction of the parent radioligand was expressed as a percent deviation from the mean 6-h value (average of 5 to 7 h). At earlier time points, the respective values for 125, 170, 215, 260, and 305 min were 41%, 17%, 7%, 3%, and 2% greater than the 6-h value (Fig. 3A), indicating that plasma levels were approaching steady state conditions by 5 h. Between 360 and 540 min the deviation was very small, -1% (Fig. 3A). Additionally, the nicotine infusion did not alter the constant plasma levels of nonmetabolized radioligand. From Fig. 3B, it can be seen that nearly 60% of the radioactivity present in the free fraction of plasma is the parent radioligand. The confirmation of constant plasma levels of nonmetabolized radioligand observed at times greater than 5 h is consistent with the stable radioactivity levels measured simultaneously in the thalamus, forebrain, and cerebellum (Fig. 2). Together these results are consistent with the development of steady state conditions.

Apparent binding potential, BP*

Calculated BP* values based on ROI analysis from the B+I studies and the high reproducibility of these measurements within animals and between brain regions are summarized in Table 1. Forebrain provided the most reproducible estimates of BP*, relative to the larger variability observed for the thalamus and cerebellum (Table 1). An example of the total radioactivity and nondisplaceable radioactivity images with the resultant subtracted image yielding specifically bound radioactivity is shown in Fig. 4. The BP* map and a fused BP* - MRI image are presented in Fig. 5. The map was created by determining the C_{SB} throughout the entire brain from a subtraction image based on the two steady state conditions used for the ROI analysis. Subsequently C_{SB} was divided by the C_{ND} determined for the forebrain ROI because it was the most accurate measure of this parameter. The high level of accuracy for the forebrain C_{ND} likely can be attributed to the brain volume being much larger than the thalamus and the cerebellum. This characteristic results in more favorable counting statistics, which is an important factor when considering that the radioactivity count is markedly decreased near the end of the study (ca. 4 half-lives from the start of the study). The magnitude of the BP* estimates is consistent with distribution and density of $\alpha 4\beta 2^*$ nAChRs in the rat brain: thalamus > forebrain > cerebellum (Table 2 and Figs. 4 and 5).

Magnitude of K_{bol} affects the onset of steady state conditions

To determine how the use of B+I paradigms with different K_{bol} can affect the onset of steady state conditions, TACs were subdivided into three groups based on K_{bol} values and graphed (Fig. 6). These data suggest that it is possible to achieve steady state conditions over shorter time periods using the B+I paradigm with 2-[¹⁸F]F-A-85380. As described above, the BP* values in Table 1 were based on an average K_{bol} of 400 min and the 300- to 420-min average total radioactivity concentration for all ROIs. If a 5% deviation from the mean is acceptable, steady state conditions for total radioactivity in all ROIs can be obtained 1 h earlier at 240 min using any of the three K_{bol} values (Fig. 6). Narrowing the focus of a study to a single ROI provides other options. If forebrain BP*s are of sole interest, steady state conditions are achievable by 105 min with a K_{bol} of 380 min (Fig. 6, top panel). With the thalamus as the primary objective, a K_{bol} of 440 min would provide steady state conditions in approximately 100 min as well (Fig. 6, bottom panel). Thus, depending on the targeted ROI, the bolus fraction of the administered radioactivity (K_{bol} value) can be adjusted to shorten the onset to attaining steady state conditions.

Effect of mass dose of the infusion on BP*

One criterion for PET radioligand studies evaluating BP* is to administer the radioligand using a tracer dose, that is, a mass dose that occupies a negligible fraction of the receptors. For BP* determinations based on steady state conditions, violating the tracer dose requirement increases receptor occupancy by the radioligand and leads to an underestimation of BP* values. Consequently, excessive receptor occupancy by 2-[¹⁸F]F-A-85380 and the underestimation of BP* will depend on increased plasma levels, which in turn are dependent on the radioligand dose. To characterize this effect, the mass dose of the radioligand infused per unit time was varied in a series of B+I experiments and BP* values were determined. As shown in Fig. 7, increasing the mass dose infusion rate results in a dose-dependent decrease in the observed BP* values. Based on these data, in order to have an underestimation of less than 5%, it is necessary to use a mass dose infusion rate of less than 0.015 nmol/kg/h.

Discussion

Our primary goal was to develop a minimally invasive method to quantify $\alpha 4\beta 2^*$ nAChRs in the rat brain using PET. This goal was accomplished by the development of a B+I paradigm for the administration of 2-[¹⁸F]F-A-85380 that incorporates the displacement of the specifically bound radioligand by nicotine. The main outcome measure from this method is BP* (Ichise et al., 2001). Importantly, the described B+I procedure facilitates the quantification of $\alpha 4\beta 2^*$ nAChRs using BP* in rat brain from several perspectives. First, the B+I paradigm achieves steady state conditions and eliminates the need for tracer kinetic modeling, thereby removing biases that are inherent in various tracer kinetic models (Carson et al., 1993; Carson et al., 1997). Second, our inclusion of a nicotine displacement component enabled quantification of BP* from a single study even when there was no appropriate reference region in the rat brain. Furthermore, the combination of B+I with displacement of specifically bound radioligand together within a single dynamic recording also eliminated head alignment problems and the necessity of arterial blood sampling. In contrast, quantification of receptors in the brain without a reference region using a simple bolus administration of radioligand requires performing two (control and blocking) studies and rapid arterial blood sampling, which can be problematic in the rat. Finally, we repeatedly imaged individual rats over a period of 5 months obtaining satisfactory replicability of our BP* measurements and demonstrating the suitability of this model for use in long-term studies. Based on these positive results, we concluded that the rat is a valid model for the *in vivo* quantification of $\alpha 4\beta 2^*$ nAChRs using microPET.

BP*, VD, and optional blood sampling

BP* values obtained in the present study using 2-[¹⁸F]F-A-85380 closely matched the pattern of receptor density distribution in the rat brain measured *in vitro* (Table 2) using a structural analogue 5-[¹²⁵I]I-A-85380 (Mukhin et al., 2000). Compared with BP* values determined with 2-[¹⁸F]F-A-85380 in rhesus monkeys or humans, values in the rat brain were consistently larger in magnitude for similar brain regions, suggesting higher densities of receptors in rats (Table 2). Indeed, using the 5-[¹²⁵I]I-A-85380 *in vitro* binding technique, it was shown that the $\alpha 4\beta 2^*$ nAChR density in the human cortex is 0.98 pmol/g tissue (Mukhin et al., 2000), which is approximately 1/4th of that in rats (Table 2).

The B+I method using 2-[¹⁸F]F-A-85380 to quantitatively determine BP* circumvents blood sampling. Yet, if it is necessary to calculate individual VD_{SB} and VD_{ND} values, we found that it is possible to obtain the necessary arterial blood samples for determination of the concentration of unmetabolized tracer from the caudal tail artery using vascular punctures. Rapid arterial sampling is routinely performed in quantitative PET studies of humans and large-bodied animals (nonhuman primates, cats, and pigs), but it is more challenging in the much

smaller rat. Even with insertion of a femoral arterial catheter, the small caliber of the vessels in rats limits the rate of collection. For example, Fujita et al. (2005) found it necessary to prolong the bolus administration in a rat study in order to acquire sufficient volumes of blood (e.g., 150 μ l over 1 min for a 6 min infusion) and plasma levels of radioactivity to characterize the input function. In B+I studies at times when steady state conditions are achieved, requirements for precise sampling times, intervals, and durations are not critical. Thus, there is the capability to assay concentrations of unmetabolized 2-[18 F]F-A-85380 in the plasma to calculate VD values.

In a few experiments where the concentrations of free nonmetabolized 2-[18 F]F-A-85380 in arterial blood plasma were measured, it was possible to calculate the VD values (Table 3). VD_{Tot} values for four different species generally covaried with BP^* values presented in Table 2. In contrast to their VD_{Tot} values, the VD_{ND} values for all brain regions for the rat and rhesus monkey were similar in magnitude within species (Table 3). However, VD_{ND} values for the isoflurane-anesthetized rat were 30% larger than the values reported for the saffan-anesthetized rhesus monkey (Table 3). Since it was shown within the same cohort of rhesus monkeys that the VD_{Tot} for the cerebellum measured in isoflurane-anesthetized animals exceeded the VD_{Tot} in saffan-anesthetized animals by 26% (Chefer et al., 2003b), it should be considered that the high VD_{ND} values in rats relative to rhesus monkeys may be related to the isoflurane anesthesia. From Table 3, it also can be seen that the VD_{Tot} of 2-[18 F]F-A-85380 for the rhesus monkey cerebellum is nearly equal to the VD_{ND} , which suggests the presence of only a small fraction of specifically bound radioactivity. It should be noted that the VD_{ND} values measured in the thalamus and forebrain of rat were very close (Table 3). These results, obtained in four animals, are consistent with the previous determination of similar VD_{ND} values for the thalamus and cortex in Rhesus monkeys (Table 3) and suggest that the VD_{ND} as well as the C_{ND} in the forebrain can be used as a measure of VD_{ND} and C_{ND} for the rat thalamus, respectively. Therefore, at equilibrium conditions, C_{ND} in the forebrain can be a good estimate of VD_{ND} in the rat thalamus.

Reference region method considerations for 2-[18 F]F-A-85380

Another alternative to measure BP^* in a single study without blood sampling and without displacement of specifically bound radioactivity is the reference region approach, which makes use of region devoid of specific binding to serve as a surrogate measure for the free radioligand concentration in other brain regions. This approach can be performed using several modeling techniques (Hume et al., 1992; Ichise et al., 2001; Ichise et al., 2003; Lammertsma et al., 1996; Logan et al., 1996), all of which employed a TAC from the reference region as the input function to calculate BP^* for the target region. While Chefer et al. (2003a) were able to use the cerebellum as a reference region in the rhesus monkey (Table 3), the present results clearly demonstrate that the cerebellum, despite having the lowest BP^* value for 2-[18 F]F-A-85380 in the rat brain (Table 1 and Fig. 5), cannot be used as a reference region to determine BP^* values. At steady state conditions, approximately 50% of the radioactivity in the cerebellum was displaced by nicotine (Fig. 2), and consequently the VD_{Tot} for 2-[18 F]F-A-85380 in cerebellum was more than twice as large as the VD_{ND} determined for any of the brain regions studied (Table 3). This result is consistent with the presence of nAChRs in the rat cerebellum, which was established more than 15 years ago (Clarke et al., 1985; Flores et al., 1992), and with the recent *in vitro* characterization of the $\alpha 4\beta 2^*$ subtype in this brain region (Turner and Kellar, 2005). The obvious problem in computing BP^* for 2-[18 F]F-A-85380 in the rat using the cerebellum as a reference region will be a serious underestimation (ca. two-fold) of BP^* , as appreciable levels of specifically bound radioactivity to $\alpha 4\beta 2^*$ nAChRs will be incorrectly included in the nonspecific radioactivity counts associated with the reference region.

Additional benefits of displacement studies

To overcome the absence of a reference region that lacks $\alpha 4\beta 2^*$ nAChRs in the rat brain, we used displacement with nicotine during the B+I administration of the radioligand to measure nondisplaceable radioactivity. Since near steady state conditions for both the brain region radioactivities and the concentration of unmetabolized 2-[^{18}F]F-A-85380 in blood were achieved at 5 h after starting the radioligand infusion (Figs. 2, 3, and 6) and the plasma concentration of unmetabolized 2-[^{18}F]F-A-85380 was not affected by the nicotine infusion (Figs. 2 and 3), the BP^* can be easily calculated as the ratio of radioactivity concentrations before nicotine challenge (C_{Tot}) and at the end of 2 h nicotine infusion (C_{ND}) minus one (see equation 4). Assuming that the VD_{ND} for 2-[^{18}F]F-A-85380 in the rat thalamus is equal to that in the forebrain (Table 3), the C_{ND} measured in forebrain was used for calculating BP^* values for both the forebrain and the thalamus. One advantage of this approach is to decrease the statistical error in measuring the C_{ND} in the thalamus. Because of the small volume of thalamus (0.03 cm^3) and the decay of ^{18}F , the real radioactivity in the entire thalamus was ca. 10 Bq at the end of the study. Consequently, the counting statistics were necessarily poor, resulting in poor precision of the estimate of C_{ND} relative to the C_{ND} estimate for determined for the much larger forebrain.

Another benefit of using the C_{ND} in forebrain as measure of that in the thalamus is the occurrence of a more complete washout of 2-[^{18}F]F-A-85380 in the forebrain during the administration of nicotine, which increases the accuracy of C_{ND} (Fig. 2). Consistent with our results, the $\text{T}_{1/2}$ values for the washout of radioactivity from the thalamus and forebrain were similar (17.3 and 17.9 min; Table 3). These half-life estimates predict that at 98 min after initiating displacement, almost 98% of the specifically bound radioactivity will be displaced and eliminated from these brain regions. Nonetheless, since the BP^* in thalamus is close to 6 (see Table 1), the residual 2% radioactivity from specific binding will result in an overestimation of C_{ND} in thalamus by 12% ($2\% \times 6$) and consequently a systematic underestimation of BP^* by 12%. The corresponding underestimation of the forebrain ($\text{BP}^* 2.6$) will be only 5% ($2\% \times 2.6$). To more completely displace the specifically bound radioactivity, and thereby achieve a more accurate estimate of the nondisplaceable radioactivity, the nicotine infusion period can be lengthened. For example, extending the displacement period by 1 h (ca. 3 additional $\text{T}_{1/2}$ values of radioactivity washout curves) to a 3-h nicotine infusion (ca. 9 $\text{T}_{1/2}$ values for radioactivity washout curves) will reduce the overestimation of C_{ND} in thalamus and forebrain to 1.5 and 0.7 % respectively. In order to meet this requirement in the course of a 9-h study, the pre-nicotine infusion period required to reach a steady state can be shortened by 1 h.

Mass dose effect

Based on studies assessing how the mass dose affected BP^* , a mass dose infusion rate of 0.29 nmol/kg/h was determined to underestimate receptor occupancy by 50% (see Fig. 7). The average ($\pm \text{SD}$) infusion rate of high specific activity 2-[^{18}F]F-A-85380 used in the present study to measure BP^* (see Table 1) was $0.032 \pm 0.013 \text{ nmol/kg/h}$ with a range of 0.017 to 0.055 nmol/kg/h. Assuming that 2-[^{18}F]F-A-85380 interacts *in vivo* with a homogeneous population of binding sites having the same apparent affinity for 2-[^{18}F]F-A-85380, the receptor occupancy attained by an infusion dose 0.032 nmol/kg/h underestimates the respective BP^* by 10% ($\text{Dose}/(\text{Dose} + \text{ED}_{50})$). In order to reduce this underestimation to a more acceptable value of less than 5%, the infusion rate consequently should be under 0.015 nmol/kg/h. This condition would require a radioactivity infusion rate of 7.5 MBq/kg/h (0.2 mCi/kg/h) and a specific activity of 500 GBq/ μmol or 13,000 Ci/mmol if the infusion was to be maintained for 9 h.

Flexibility in B+I study design

In the current study, the duration of the pre-nicotine challenge period was set at 7 h in order to provide ample time to firmly establish the earliest time at which steady state conditions developed and to evaluate the feasibility of using alternative protocol designs using the displacement technique. Having shown that steady state conditions are achieved by 5 h, it is possible within the framework of a 9-h study to use an additional intervention (i.e., a partial displacement of radioligand starting at 5 h) before initiating the complete displacement of specifically bound radioactivity at 7 h. Designs for shorter studies also are possible. If BP* is the only measurement needed for several brain regions and if a 5% bias is acceptable, the nicotine displacement could be initiated at 4.5 h of the 2-[¹⁸F]F-A-85380 infusion (see Fig. 6, middle panel). In this case, the total radioactivity can be obtained by averaging frames from 3.5 to 4.5 h and the duration of the study can be reduced to 6.5 to 7.5 h. Alternatively, if the BP* of interest is restricted to one specific area of the brain such as forebrain and the appropriate K_{bol} (see Fig. 6, middle panel) is selected, the measurement of C_{Tot} can be initiated as early as 2 h after beginning radioligand administration. With the inclusion of a displacement period, the total duration of a forebrain study should be approximately 5 to 6 hours. Note that between 2 to 4 h, a true steady state will not be achieved because the concentration of nonmetabolized 2-[¹⁸F]F-A-85380 in plasma will continue to decrease (see Fig. 3). Nonetheless, stable radioactivity in any brain area at any time beyond 2 hours can provide the measurement for C_{Tot} at equilibrium. In this situation, if the object is to calculate VD_{Tot}, the plasma radioactivity concentration of free radioligand could be measured at a few time points near the end of the study (i.e., 5 to 6 h after starting the 2-[¹⁸F]F-A-85380 infusion), when true steady state conditions are achieved (see Fig. 3). Based on the experimental aims and the brain area of interest, the basic B+I and displacement methodology we characterized can provide flexibility in designing other experimental protocols.

Contending with high specific activity requirements

The requirement for high specific activities for the B+I method applied to small animals is a consequence of the low sensitivity of the scanner, small brain volume, and study length. Still, producing such specific activities should not be considered unduly formidable for a radiochemistry laboratory or using this method in animal or human studies. An easy adjustment would be to shorten the length of the experiment as suggested previously. For example, constraining the mass dose to 0.015 nmol/kg/h and decreasing the scanning time by 2 h would permit the specific activity needed to be reduced by 50%. Optimizing the radiosynthesis procedures is also feasible. Schmaljohann et al. (2005) have described a safe, fast automated production of 2-[¹⁸F]F-A-85380, which yielded specific activities of > 300 GBq/μmol within 35 min (0.3 half-life of ¹⁸F). This is a considerably shorter time than the 120 min (1.1 half-life) required by the method we used (Horti et al., 1998). Until recently, the lowest acceptable specific activity for our human studies (Kimes et al., 2006a,b) was 240 GBq/μmol (6500 Ci/mmol), which used ca. 0.2 Ci of initial ¹⁸F radioactivity. Increasing the amount of initial radioactivity to ca. 1 Ci yielded specific activities that regularly exceeded 400 GBq/μmol (upper limit 1251 GBq/μmol). The increased efficiency of these modifications, used alone or possibly in combination, should facilitate the acquisition of high specific activity radioligand production.

Even though our focus has been on small animal PET studies, it should be recalled that the requirement for high specific activity for human studies is less critical because of higher scanner sensitivity and larger brain volumes. We have found it practical to use a single bolus dose of 2-[¹⁸F]F-A-85380 over 8 h with a smaller dose of radioactivity (4 to 5 mCi/70 kg), but this requires arterial catheterization for blood sampling (Kimes et al., 2006a). The B+I procedure has been successfully used in humans with 2-[¹⁸F]F-A-85380 (Brody et al., 2006; Kimes et al., 2006b) as well. An important feature of these human B+I studies was the use of radioactivity

doses comparable to those of the single bolus human studies. Similar to the present rodent study and in contrast to single bolus human studies, producing steady state conditions in human PET studies with the B+I method affords the benefit of enabling intravenous catheterization to supplant arterial catheterization to acquire blood samples for radioligand analysis (Kimes et al., 2006a).

Conclusion

The B+I administration of 2-[¹⁸F]F-A-85380 can be successfully used to quantify $\alpha 4\beta 2^*$ nAChR levels *in vivo* in the rat brain with good reproducibility. Compared with bolus methods, this BP* quantification method is relatively simple because neither tracer kinetic modeling nor arterial blood samples are required. Although 2-[¹⁸F]F-A-85380 is characterized by slow kinetics, such that complete B+I studies with a displacement component must last 5 to 7 h, this approach can be routinely successful, and we were able to image individual rats repeatedly over a period of six months. The successful repeated application of the B+I method for the quantification of receptors provides the opportunity to study long-term changes in $\alpha 4\beta 2^*$ nAChRs in the rat brain and gain the advantage of a within-subjects design.

Acknowledgements

This work was supported by the Intramural Research Program of the National Institute on Drug Abuse. The invaluable contributions of Ms. Amy L. Kunce of the Neuroimaging Research Branch and David L. Bailey of Siemens-Concorde to expand the operational capability of the microPET to acquire 9-hour imaging studies are gratefully appreciated. The authors also acknowledge the expertise of Mr. Dean Shumway in analyzing blood samples, Dr. Hanbing Lu and Mr. William Rea in acquiring the rat MRI images, and Ms. Susan S. Vaupel, Institute for Laboratory Animal Research, The National Academies, for editorial assistance.

Appendix A. Terms

BP*	Apparent binding potential: defined as the quotient of C _{Tot} divided by C _{ND} minus 1 or the quotient of VD _{SB} divided by VD _{ND} .
C _P	Concentration of plasma radioactivity from free nonmetabolized radioligand.
C _{SB}	Concentration of specifically bound radioactivity in tissue.
C _{ND}	Concentration of nondisplaceable radioactivity: both free and nondisplaceable radioactivity in tissue.
C _{Tot}	Concentration of total radioactivity: specifically bound, nondisplaceable, and free radioactivity in tissue.
F	Concentration of free nonmetabolized radioligand in blood plasma.
VD _{SB}	Volume of distribution of the specifically bound radioactivity compartment.
VD _{ND}	Volume of distribution of the nondisplaceable radioactivity compartment, which includes both free and nonspecifically bound radioactivity.
VD _{Tot}	Volume of distribution of the total radioactivity compartment, which includes specifically bound, nonspecifically bound, and free radioactivity.

References

Bottlaender M, Valette H, Roumenov D, Dollé F, Coulon C, Ottaviani M, Hinnen F, Richard M. Biodistribution and radiation dosimetry of ¹⁸F-fluoro-A-85380 in healthy volunteers. *J. Nuc. Med* 2003;44:596–601.

Brody AK, Mandelkern MA, London ED, Olmstgead RE, Farahi J, Scheibal D, Jou J, Allen V, Tionsgon E, Chefer SI, Koren AO, Mukhin AG. Cigarette smoking saturates brain $\alpha 4\beta 2$ nicotinic acetylcholine receptors. *Arch. Gen. Psychiatry* 2006;63:907–915. [PubMed: 16894067]

Carson RE, Channing MA, Blasberg RG, Dunn BB, Cohen RM, Rice KC, Herscovitch P. Comparison of bolus and infusion methods for receptor quantitation: application to [¹⁸F]-cyclofoxy and positron emission tomography. *J. Cereb. Blood Flow Metab* 1993;13:24–42. [PubMed: 8380178]

Carson RE, Breier A, de Bartolomeis A, Saunders RC, Su TP, Schmall B, Der MG, Pickar D, Eckelman WC. Quantification of amphetamine-induced changes in [¹¹C]raclopride binding with continuous infusion. *J. Cereb. Blood Flow Metab* 1997;17:437–447. [PubMed: 9143226]

- Chefer SI, London ED, Koren AO, Pavlova OA, Kurian V, Kimes AS, Horti AG, Mukhin AG. Graphical analysis of 2-[¹⁸F]FA binding to nicotinic acetylcholine receptors in rhesus monkey brain. *Synapse* 2003a;48:25–34. [PubMed: 12557269]
- Chefer, SI.; Pavlova, OA.; Horti, AG.; Hall, A.; Kurian, V.; Mukhin, AG. Pharmacokinetics and in vivo receptor binding of 2-[¹⁸F]F-A-85380 under isoflurane and saffan anesthesia; Society for Neuroscience, 33rd Annual Meeting; Washington, DC. Abstract Viewer/Itinerary Planner. 2003b. Abstr. 683.11.
- Clarke PBS, Schwartz RD, Paul SM, Pert CB, Pert A. Nicotinic binding in rat brain: autoradiographic comparison of [³H]acetylcholine, [³H]nicotine, and [¹²⁵I]- α -Bungarotoxin. *J. Neurosci* 1985;5:1307–1315. [PubMed: 3998824]
- Defrise M, Kinahan PE, Townsend DW, Michel C, Sibomana M, Newport DF. Exact and approximate rebinning algorithms for 3-D PET data. *Transactions on Medical Imaging, IEEE* 1997;16:145–158.
- Ding Y-S, Molina PE, Fowler JS, Logan J, Volkow ND, Kuhar MJ, Carroll FI. Comparative studies of epibatidine derivatives [¹⁸F]NFEP and [¹⁸F]N-methyl-NFEP: kinetics, nicotine effect, and toxicity. *Nucl. Med. Biol* 1999;26:139–148. [PubMed: 10096514]
- Flores CM, Rogers SW, Pabreza LA, Wolfe BB, Kellar KJ. A subtype of nicotinic cholinergic receptor in rat brain is composed of $\alpha 4$ and $\beta 2$ subunits and is upregulated by chronic nicotine treatment. *J. Pharmacol. Exp. Ther* 1992;41:31–37.
- Fujita M, Zoghbi SS, Crescenzo MS, Hong J, Musachio JL, Lu J-Q, Liow J-S, Seneca N, Tipre DN, Cropley VL, Imaizumi M, Gee AD, Seidel J, Green MV, Pike VW, Innis RB. Quantification of brain phosphodiesterase 4 in rat with (R)-[¹¹C]rolipram-PET. *NeuroImage* 2005;26:1201–1210. [PubMed: 15961054]
- Gallezot J-D, Bottlaender M, Grégoire M-C, Roumenov D, Deverre J-R, Coulon C, Ottaviani M, Dollé F, Syrota A, Valette H. In vivo imaging of human cerebral nicotinic acetylcholine receptors with 2-¹⁸F-fluoro-A-85380 and PET. *J. Nuc. Med* 2005;46:240–247.
- Holladay MW, Dart MJ, Lynch JK. Neuronal nicotinic acetylcholine receptors as targets for drug discovery. *J. Med. Chem* 1997;40:4169–4194. [PubMed: 9435889]
- Horti AG, Koren AO, Ravert HT, Musachio JL, Mathews WB, London ED, Dannals RF. Synthesis of a radiotracer for studying nicotinic acetylcholine receptors: 2-[¹⁸F]fluoro-3-(2(S)-azetidinylmethoxy)pyridine (2-[¹⁸F]A-85380). *J. Labelled Cpd. Radiopharm* 1998;41:309–318.
- Hume SP, Myers R, Bloomfield PM, Opacka-Juffry J, Cremer JE, Ahier RG, Luthra SK, Brooks DJ, Lammertsma AA. Quantitation of carbon-11-labeled raclopride in rat striatum using positron emission tomography. *Synapse* 1992;12:47–54. [PubMed: 1411963]
- Ichise M, Meyer JH, Yonekura Y. An introduction to PET and SPECT neuroreceptor quantification models. *J. Nucl. Med* 2001;42:755–763. [PubMed: 11337572]
- Ichise M, Liow J-S, Lu J-Q, Takano A, Model K, Toyama H, Suhara T, Suzuki K, Innis RB, Carson RE. Linearized reference tissue parametric imaging methods: application to [¹¹C]DASB positron emission tomography studies of the serotonin transporter in human brain. *J. Cereb. Blood Flow Metab* 2003;23:1096–1112. [PubMed: 12973026]
- Kimes AS, Horti AG, London ED, Chefer SI, Contoreggi C, Ernst M, Friello P, Koren AO, Kurian V, Matochik JA, Pavlova O, Vaupel DB, Mukhin AG. 2-[¹⁸F]F-A-85380: PET imaging of brain nicotinic acetylcholine receptors and whole body distribution in humans. *FASEB J* 2003;17:1331–1333. [PubMed: 12759330]Epub 2003 May 20.
- Kimes AS, Chefer SI, Matochik JA, Pavlova OA, Contoreggi CS, Horti AG, Kurian V, Vaupel DB, Koren AO, Ernst M, London ED, Mukhin AG. 2004. PET quantitation of nicotinic acetylcholine receptors in the human brain. *NeuroImage* 2004;22(Suppl 2):T79.
- Kimes AS, Chefer S, Contoreggi CS, Hall A, Horti A, Vaupel DB, Pavlova O, Stein EA, Mukhin AG. In vivo quantification of the greater densities of $\alpha 4 \beta 2^*$ nicotinic acetylcholine receptors in smokers compared to non-smokers. *NeuroImage* 2006a;31(Suppl 2):T153.
- Kimes AS, Chefer S, Contoreggi CS, Vaupel DB, Stein EA, Mukhin AG. Quantification of $\alpha 4 \beta 2^*$ nicotinic acetylcholine receptors in human brain using bolus plus infusion administration of 2-[F-18] F-A-85380. *NeuroImage* 2006b;31(Suppl 2):T102.

- Koren AO, Horti AG, Mukhin AG, Gündisch D, Kimes AS, Dannals RF, London ED. 2-, 5-, and 6-Halo-3-(2(*S*)-azetidinylmethoxy)pyridines: synthesis, affinity for nicotinic acetylcholine receptors, and molecular modeling. *J. Med. Chem* 1998;41:3690–3698. [PubMed: 9733494]
- Lammertsma AA, Bench CJ, Hume SP, Osman S, Gunn K, Brooks DJ, Frackowiak RSJ. Comparison of methods for analysis of clinical [¹¹C]raclopride studies. *J. Cereb. Blood Flow Metab* 1996;16:42–52. [PubMed: 8530554]
- Le Foll B, Goldberg SR. Nicotine as a typical drug of abuse in experimental animals and humans. *Psychopharmacology* 2006;184:367–381. [PubMed: 16205918]
- Lindstrom J, Anand R, Peng X, Gerzanich V, Wang F, Li Y. Neuronal nicotinic receptor subtypes. *Ann. NY. Acad. Sci* 1995;757:100–116. [PubMed: 7611667]
- Logan J, Fowler JS, Volkow ND, Wang G-J, Ding Y-S, Alexoff DL. Distribution volume ratios without blood sampling from graphical analysis of PET data. *J. Cereb. Blood Flow Metab* 1996;16:834–840. [PubMed: 8784228]
- Mitkovski S, Villemagne VL, Kovakovic KE, O'Keefe G, Tochon-Danguy H, Mulligan RS, Dickinson KL, Saunderson T, Gregoire M-C, Bottlaender M, Dolle F, Rowe CC. Simplified quantification of nicotinic receptors with 2[¹⁸F]F-A-85380 PET. *Nuc. Med. Biol* 2005;32:585–591.
- Molina PE, Ding Y-S, Carroll FI, Liang F, Volkow ND, Pappas N, Kuhar M, Abumrad N, Gately SJ, Fowler JS. Fluro-norchloroepibatidine: preclinical assessment of acute toxicity. *Nucl. Med. Biol* 1997;24:743–747. [PubMed: 9428600]
- Mukhin AG, Gündisch D, Horti AG, Koren AO, Tamagnan G, Kimes AS, Chambers J, Vaupel DB, King SL, Picciotto MR, Innis RB, London ED. 5-Iodo-A-85380, an $\alpha 4\beta 2$ subtype-selective ligand for nicotinic acetylcholine receptors. *J. Pharmacol. Exp. Ther* 2000;273:642–649.
- Pavlova, OA.; Gündisch, D.; Koren, AO.; Horti, AG.; Chefer, SI.; Kimes, AS.; Mukhin, AG.; London, ED. In vitro characterization of 2-[¹⁸F]F-A-85380, a novel PET ligand for $\alpha 4\beta 2$ nicotinic receptors; Society for Neuroscience 30th Annual Meeting; New Orleans, La.. 2000. Abstr. 26, 627, 235.16.
- Paxinos, G.; Huang, X-F.; Toga, AW. The rhesus monkey brain in stereotaxic coordinates. Academic Press; New York: 2000.
- Schmaljohann J, Gündisch D, Minnerop M, Joe A, Bucerius J, Dittmar C, Jessen F, Gohlke S, Wüllner U. A simple and fast method for the preparation of n.c.a. 2-[¹⁸F]F-A-85380 for human use. *Appl. Rad. Iso* 2005;63:433–435.
- Shumway D, Pavlova O, Mukhin A. Simplified measurement of 2-[¹⁸F]F-A-85380 parent fraction in blood plasma during PET studies. *NeuroImage* 2006;2006;31(Suppl 2):T44.
- Tai YC, Chatziioannou A, Siegel S, Young J, Newport D, Goble RN, Nutt RE, Cherry SR. Performance evaluation of the microPET P4: a PET system dedicated to animal imaging. *Phys. Med. Biol* 2001;46:1845–1862. [PubMed: 11474929]
- Turner JR, Kellar KJ. Nicotinic cholinergic receptors in the rat cerebellum: multiple heteromeric subtypes. *J. Neurosci* 2005;25:9258–9265. [PubMed: 16207885]
- Valette H, Bottlaender M, Dollé F, Guenther I, Fuseau C, Coulon C, Ottaviani M, Crouzel C. Imaging central nicotinic acetylcholine receptors in baboons with [¹⁸F]fluoro-A-85380. *J. Nuc. Med* 1999;40:1374–1380.
- Valette H, Bottlaender M, Coulon C, Ottaviani M, Syrota A. Anesthesia affects the disposition of [¹⁸F]fluoro-A-85380: a PET study in monkeys. *Synapse* 2002;44:58–58. [PubMed: 11842447]
- Vaupel DB, Tella SR, Huso DL, Mukhin AG, Baum I, Wagner VO, 3rd Horti, A.G. London ED, Koren AO, Kimes AS. Pharmacology, toxicology, and radiation dosimetry evaluation of [¹²³I]5-I-A-85380, a radioligand for in vivo imaging of cerebral neuronal nicotinic acetylcholine receptors in humans. *Drug Dev. Res* 2003;58:149–168.
- Vaupel DB, Tella SR, Huso DL, Wagner VO 3rd, Mukhin AG, Chefer SI, Horti AG, London ED, Koren AO, Kimes AS. Pharmacological and toxicological evaluation of 2-fluoro-3-(2(*S*)-azetidinylmethoxy)pyridine (2-F-A-85380), a ligand for imaging cerebral nicotinic acetylcholine receptors with positron emission tomography. *J. Pharmacol. Exp. Ther* 2005;312:355–65. [PubMed: 15331657]Epub 2004 Aug 26.

Figure 1.

Time-activity curves after a single intravenous bolus administration of 2- ^{18}F -A-85380 from a single rat study. Similar results were obtained in two additional studies. Based on these time-activity curves, a K_{bol} of 400 min was predicted to be optimal for the bolus-plus-infusion studies. The dose of radioligand was administered over a period of 1 min. The forebrain region consists of the brain tissue rostral to a coronal plane passing between the inferior and superior colliculi extending ventrally through the pons, thereby excluding the cerebellum, medulla, and brain stem.

Figure 2.

The intravenous bolus-plus-infusion paradigm with 2-[^{18}F]F-A-85380 achieved steady state levels in the rat brain and incorporated a nicotine displacement component. The time activity curves for 2-[^{18}F]F-A-85380 radioactivity in the thalamus (●), forebrain (■), and cerebellum (○) are shown for a 9-h study using a $K_{\text{bol}} = 400$ min. Once steady state concentrations of radioactivity in the brain were attained (5 to 7 h), unlabeled nicotine was administered intravenously via a second lateral tail vein over 2 h to effectively displace specifically bound radioligand within that time period. Plasma levels of nonmetabolized 2-[^{18}F]F-A-85380 (▲) were determined from blood samples taken from the caudal tail artery. The data indicate steady state levels of free unmetabolized radioligand were attained at approximately 5 h. BP^* values for regions of interest were calculated based on the ratio of the concentrations of total radioactivity to nondisplaceable radioactivity minus 1.

Figure 3.

Steady state plasma levels of nonmetabolized 2-[¹⁸F]F-A-85380 were not affected by a 2-h infusion of unlabeled nicotine. The time-activity curve for arterial plasma levels of the free radioligand (▲) is based on 8 studies, which had a mean K_{bol} value of 400 min ($n=3$ for 380 min, $n=2$ for 440 min, $n=1$ for 420, 410 and 350 min). Values are the means $\pm$ SD. Nicotine was infused at a rate of 0.4 mg/kg/h for 15 min and 0.1 mg/kg/h for 105 min. The bar graph insert shows the parent (nonmetabolized) 2-[¹⁸F]F-A-85380 fraction relative to total radioactivity in plasma ($47 \pm 3\%$; mean $\pm$ SEM; $n=4$) measured at 7 h and the fraction of the free (not bound to plasma proteins) 2-[¹⁸F]F-A-85380 in rat plasma ($56 \pm 3\%$; $n=3$).

Figure 4.

MicroPET images of the total (C_{Tot}) and the nondisplaceable (C_{ND}) radioactivity concentrations and the corresponding subtraction images for specifically bound radioactivity (C_{SB}) in the rat brain were derived from a 9-h bolus-plus-infusion study with a pharmacological displacement of specifically bound 2- $[^{18}\text{F}]$ F-A-85380 by nicotine. (A) coronal view; (B) sagittal view; (C) horizontal view. The total binding image was obtained at steady state conditions, which were present during the 5 to 7-h period of the constant infusion. At 7 h into the study, unlabeled nicotine was infused over a 2-h period and the nondisplaceable radioactivity image was acquired during the last 45 min of the nicotine infusion when a second steady state was approached. The pattern of distribution of specifically bound radioactivity and BP^* values was consistent with that of $\alpha 4\beta 2^*$ nicotinic receptors in the rat brain.

Figure 5.

A. The upper row shows anatomical T 1-weighted MRI images of a Sprague-Dawley rat for coronal, sagittal, and horizontal slices. B. The middle row shows the apparent binding potential (BP*) images based on the average of four animals that are coregistered with the MRI slices. The BP* parametric map was created by determining the specifically bound radioactivity concentrations throughout the entire brain and dividing these values by the concentration of nondisplaceable radioactivity determined for the forebrain region of interest. C. The bottom row presents fused PET/MRI images.

Figure 6.

Effect of K_{bol} on acquisition of steady state conditions in different regions of interest. Steady state baselines for each region of interest are operationally defined as the mean radioactivity concentrations measured at 6 h (average of 5- to 7-h values). All concentrations are expressed as a percent of the respective steady state baseline to provide a means of comparing the attainment of steady state conditions based upon percent changes from baseline.

Figure 7.

Increasing the mass dose infusion rate caused a dose-dependent underestimation of BP* values. The curves above represent the results of 17 separate bolus-plus-infusion studies performed in 4 rats. Observed BP* values were expressed as a percent of the true BP*. The ED_{50} is estimated to be 0.29 nmol/kg/h. The insert illustrates a typical s-shaped curve when the same data were replotted using a log dose scale.

Table 1Average value, variability, and reproducibility of 2- ^{18}F F-A-85380 BP* measurement in rat brain.

	Thalamus	Forebrain	Cerebellum
Rat 1, n=3			
Average BP* $\pm$ SD	4.1 $\pm$ 0.4	1.8 $\pm$ 0.2	0.8 $\pm$ 0.1
CV	9%	9%	18%
Rat 2, n=3			
Average BP* $\pm$ SD	5.9 $\pm$ 0.3	2.7 $\pm$ 0.2	0.9 $\pm$ 0.1
CV	5%	7%	8%
Rat 3, n=2			
Average BP* $\pm$ SD	7.6 $\pm$ 0.07	3.5 $\pm$ 0.03	1.4 $\pm$ 0.10
CV	1%	1%	7%
Rat 4, n=4			
Average BP* $\pm$ SD	6.1 $\pm$ 1.70	2.5 $\pm$ 0.24	1.0 $\pm$ 0.22
CV	28%	10%	22%
Unweighted average BP*	5.94	2.62	1.03
SEM	0.72	0.37	0.12
Weighted CV	13%	7%	15%

Four male Sprague-Dawley rats of identical age were imaged at repeated intervals using the bolus-plus-infusion administration of 2- ^{18}F F-A-85380 with a nicotine displacement component. The number (n) denotes the number of studies performed with the same animal, and CV is the coefficient of variation.

Table 2*In vitro* and *in vivo* quantification of $\alpha 4\beta 2^*$ nAChRs in brain regions of different species.

Species Measure Method/ Analysis	Rat ^A Receptor density <i>in vitro</i> binding	Rat ^B BP* microPET Bolus+Infusion $\frac{C_{Tot}}{C_{ND}} - 1$	Rhesus monkey ^C BP* PET Bolus Graphical analysis	Human ^D BP* PET Bolus Reference region Corpus callosum
Thalamus	5.9 fmol/mg	5.94 ± 0.72	2.00 ± 0.25	2.2
Cortex	3.8 fmol/mg	----	0.41 ± 0.07	0.3
Forebrain	3.9 fmol/mg	2.62 ± 0.37	-----	----
Cerebellum	1.3 fmol/mg	1.03 ± 0.12	0.03 ± 0.02	0.6
n	10 pooled	4	4	6

The estimation of the density of $\alpha 4\beta 2^*$ nAChRs determined *in vivo* with microPET compares favorably the *in vitro* receptor density. Based on a comparison of BP* values, the receptor densities in the rat brain were greater than those of a nonhuman and human primates.

^A Assays used 5-[¹²⁵I]-A-85380, a structural analogue of 2-[¹⁸F]-A-85380, for the radioligand and tissue from Fischer rats (Mukhin et al., 2000). B_{max} values are reported as fmol/mg tissue.

^B Isoflurane-anesthetized Sprague-Dawley rats were used for microPET studies.

^C Saffan-anesthetized rhesus monkeys (Chefer et al., 2003a).

^D Human studies (Kimes et al., 2004). All values are means based on the corresponding number (n), and variability estimates for the rat and rhesus monkey means are the SEM.

Table 3
Total and nondisplaceable volumes of distribution for 2-[¹⁸F]F-A-85380 among species.

	Human ^A VD _{Tot}	Baboon ^B VD _{Tot}	Rhesus ^C VD _{Tot}	VD _{ND}	VD _{Tot}	Rat ^D VD _{ND}	T1/2 min
Thalamus *	15 ± 2	14	10.5	3.5	33 ± 3	4.7 ± 0.2	17.3 ± 1.9
Forebrain	-----	-----	-----	-----	18 ± 2	4.8 ± 0.1	17.9 ± 1.7
Cortex	6.2 ± 0.2	8	5.2	3.7	-----	-----	-----
Cerebellum	7.0 ± 0.4	6	3.4	3.3	10 ± 1	4.3 ± 0.3	15.1 ± 1.6

Total volumes of distribution (VD_{Tot}) and nondisplaceable volumes of distribution (VD_{ND}) for 2-[¹⁸F]F-A-85380 from:

A humans (Gallezot et al., 2005),

B isoflurane-anesthetized baboons (Valette et al., 2002),

C Saffian-anesthetized rhesus monkeys (Chefer et al., 2003a),

D isoflurane-anesthetized rats (present study).

* Forebrain consists of all brain tissue rostral to the cerebellum and pons. T1/2 values are the half-lives for the nicotine-induced washout of radioactivity during the displacement phase. Data values are the means ± SEM.