PET radiotracers for molecular imaging in the brain: Past, present and future

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Abstract

Neuroimaging of brain receptors began in the early 1980s. Now, some thirty-five years later, PET imaging is still an expanding field of preclinical and clinical investigations. In addition to improvements in PET cameras and image analysis, the availability of suitable radiotracers is a crucial factor leading this expansion. Radiotracers have been developed to visualize and quantify a growing numbers of brain receptors, transporters, enzymes and other molecular targets. The development of adequate PET radiotracers represents an exciting challenge, given the large number of targets and neurochemical functions that have yet to be explored. In this article, we review the main evolutions led by preclinical radiotracers and clinical radiopharmaceuticals. The current main contributions of PET radiotracers are described in terms of imaging of neuronal metabolism, receptor and transporter quantification and neurodegenerative, neuroinflammatory and neurooncologic process imaging. In the last part, we highlight some applications presenting a potential for novel functional explorations of the brain.

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Contents

Introduction ................................................................ 363
From nuclear medicine to molecular imaging .............................................. 364
From radiochemicals to radiopharmaceuticals .............................................. 364
Current contributions of PET in brain molecular imaging ........................................... 364
Neuronal metabolism .............................................................................. 364
Brain receptors and transporters .................................................... 365
Neurodegenerative processes ...................................................... 365
Parkinson’s disease .............................................................................. 365
Alzheimer’s disease .............................................................................. 365
Neuroinflammatory processes ..................................................... 365
Neurooncologic processes .......................................................... 366
Future of PET molecular imaging: promise and pitfalls ............................................ 366
PET radiotracers for measuring new brain targets ............................................ 366
PET radiotracers for measuring endogenous neurotransmitter release ................................... 366
PET radiotracers for measuring the specific coupling of G protein-coupled receptors with agonists ................................... 366
PET radiotracers for measuring receptor internalization ......................................... 367
PET radiotracers for measuring P-glycoprotein function ......................................... 367
PET radiotracers for measuring biomarkers during drug development ................................... 367
PET radiotracers for measuring in a multimodality manner? ....................................... 368
Instrumental multimodality .............................................................. 368
Tracer multimodality, a myth? .............................................................. 368
Conclusions ................................................................ 368
References ................................................................. 368

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Introduction
From nuclear medicine to molecular imaging

The visual representation of in vivo administered radiopharmaceuticals was in the early 60s a major milestone in the nuclear medicine history. From there, nuclear medicine has relied on simultaneous advances in instrumentation and in imaging probes. Single photon emission computed tomography (SPECT) was the first imaging modality developed and the radiopharmaceuticals were mainly based on the easily available $^{99m}$Tc radioisotope (Schwochau, 1994). These $^{99m}$Tc generators (half-life of 66 h) produced an isotope, which, at that time, relied mostly on the nuclear fission reaction. With very interesting physical decay characteristics, such as a six hour half-life and a gamma emission of 141 keV, many $^{99m}$Tc radiotherapeutics were developed. Their advantageous biodistribution and selective organ accumulation, which characterized the most routinely clinically used $^{99m}$Tc radiopharmaceuticals, are mainly due to the physico-chemical properties such as lipophilicity, molecule size, electrical charge, ionic nature, complex stability, high specific activity etc. Due to the ionless nature of these radiopharmaceuticals, a few of them were successfully designed for brain exploration and the major application was blood flow imaging.

Along with the development of positron emission tomography (PET) instrumentation and the associated radiopharmaceuticals in the 1970s, researchers have widened the possible applications with the quantification of radioisotope concentration. This therefore allows kinetic modeling of the radiopharmaceuticals and extraction of in vivo physiological parameters (Phelps, 2000; Phelps and Mazziotta, 1985). PET radiopharmaceuticals were based on four major, cyclotron produced, radioisotopes ($^{15}$O, $^{13}$N, $^{11}$C, $^{18}$F which are beta $^-$ emitters only with half life of 2, 10, 20.3, 110 min, respectively). $^{15}$O labeled water was mainly used for quantitative cerebral blood flow measurement in the 1980s before its replacement by a non-radioactive method, the Bold MRI technique (Rees et al., 1997). $^{11}$C isotope offers the capacity to be incorporated, through a covalent bond, in biomolecules of interest without altering their biological properties (for example, amino acids, and neurotransmitters). $^{11}$C and $^{18}$F labeled specific probes with a recognition pattern for enzymes or receptors among others were designed and validated in vivo; their discovery opened the new field we call today “molecular imaging”. Molecular imaging aims to integrate patient-specific and disease-specific molecular information derived from diagnostic imaging studies (Jaffer and Weissleder, 2005). The goal of molecular imaging is the non-invasive visualization and quantification of molecular entities (e.g., in the brain) and its correlation to pathophysiological events. Consequently, nuclear medicine, including PET and SPECT, and its radiotracers, ranging from radiochemicals to radiopharmaceuticals, can be regarded as contributing to molecular imaging.

From radiochemicals to radiopharmaceuticals

PET (with $^{11}$C and $^{18}$F) and SPECT (with $^{123}$I, half life of 13 h) are considered presently as the most sensitive techniques for in vivo imaging of these ligand–receptor interactions with picomolar to femtomolar levels detected. Radiochemists have developed and continue to develop specific chemical pathways for the synthesis of $^{11}$C and $^{18}$F compounds taking into account: (i) the nature of the cyclotron produced molecule supporting the radioisotope (carbon dioxide for $^{13}$C and fluoride anion for $^{18}$F); (ii) the short half life of the radioisotopes, requiring a total synthesis time no longer than 1 h for $^{11}$C and 4 h for $^{18}$F; (iii) the high specific activity of the radioisotopes which has to be kept as high as possible during the synthetic process, avoiding dilution with non-labeled molecules (atmospheric carbon dioxide for $^{13}$C, fluoride present in the polymers used in the tubing or vials and in the reagents for $^{18}$F); and (iv) the toxicity of some reagents used in organic chemistry (solvents, heavy metals, etc.). As a result of all these constraints, only a limited number of radioisynthetic strategies (for more information, see Ametamey et al., 2008; Cai et al., 2008; Fowler and Wolf, 1997; Miller et al., 2008) are used nowadays for labeling the new imaging probes; these methods include electrophilic reactions with labeled methyl iodide for $^{11}$C and nucleophilic radiofluorinations of aliphatic or aromatic derivatives for $^{18}$F.

For years, in order to protect chemists from ionizing radiation, most of the new radiopharmaceuticals have been produced in homemade automatic systems. Production of radiolabeled fluorodeoxyglucose ([$^{18}$F]FDG, etc.) is the most widely used clinical PET radiotracer) has relied for more than twenty years on dedicated commercial automated systems. Today, many commercial companies are also offering automated synthesizers in which different modules are implemented, as evaporating solvents, heating solutions, mixing solutions, extracting compounds, chromatographic purification and formulation of injected solution. An additional feature has been recently incorporated by some companies: the single use cassette, avoiding cleaning and possible cross-contamination. The advantages of such systems are obvious in terms of control and repeatability of the process, handling large amounts of radioactivity and certainly easing regulatory compliance.

All radiotracers used for the purpose of diagnosis or therapy have been defined as radioactive drugs or radiopharmaceuticals by the international and national drug administrations. If, from the standpoint of chemistry and radiochemical purity, there is no difference between the terms radiochemical and radiopharmaceutical, from a regulatory point of view, a radiopharmaceutical must be sterile, pyrogen-free, according to the same specificities as for injectable drugs (Verbruggen et al., 2008). Only a few PET radiopharmaceuticals have a market authorization ($^{18}$F-FDG, etc.); the others, which are used in clinical research, rely on in-house production. Competent authorities require now that all these radiopharmaceuticals should be produced under the Good Manufacturing Practices (GMP) conditions, considerably slowing the discovery process in imaging centers. Nevertheless, an automated synthetic module, such as the one we have described earlier, with a limited number of standardized functionalities (labeling reaction, solvent elimination, deprotection reaction, chromatographic purification and radiopharmaceutical formulation), will help the faster development of new radiopharmaceuticals and will reduce dramatically the associated paperwork.

Current contributions of PET in brain molecular imaging

Neuronal metabolism

PET provides insight into energy metabolism in vivo by quantifying glucose consumption, cerebral perfusion and oxygen consumption. In neuroscience research, this allows the study of neural activity, as well as disease processes, based on the brain’s metabolism and function. The deoxyglucose technique, by employing autoradiography using radiolabeled deoxyglucose ([$^{14}$C]DG), opened the way to measure regional cerebral glucose metabolism (Sokoloff et al., 1977). The development of [$^{18}$F]FDG, a fluorine-18-labeled glucose analog used in positron emission tomography added a major dimension to the investigation of brain function.

The mechanism of [$^{18}$F]FDG brain capture is well-known. It is transported into the cell, phosphorylated and, unlike glucose, cannot be metabolized further, remaining trapped within the cytoplasm. Therefore, it allows the study of glucose transport and phosphorylation but not its subsequent metabolism. While it is recognized that glucose metabolism and cerebral blood flow probably correspond to changes in neurotransmitter function (Barros et al., 2005), cellular and molecular mechanisms that underlie the [$^{18}$F]FDG-based PET imaging are not totally known, particularly in terms of astrocyte contribution (Pellerin et al., 2007) and of neurovascular coupling (Shih et al., 2011).

In the late 1970s and early 1980s, the [$^{18}$F]FDG was widely used to examine a variety of disorders related to seizures, cerebral vascular...
Brain receptors and transporters

A primary goal of brain neurotransmitter imaging is to visualize the receptors and transporters for the major neurotransmitters involved in neurophysiological and pathological processes. The outcome measures are the density of receptors and the binding affinity by which the radioligand binds to the receptor of interest. PET ligands are radiolabeled with a high specific activity that allows a very low amount of ligand to be injected (“tracer dose”), thus minimizing any pharmacological effect. At high specific radioactivity, the maximum bound/free ratio (B/F) ratio will be equivalent to the receptor concentration/ligand affinity (Bmax/Kd). This estimation is particularly important when targeting low-density binding sites (e.g., receptors at picomolar levels), requiring radiotracers with sub-picomolar binding affinities. This notion is crucial in preclinical studies using microPET cameras: there is a balance between the injected radioactivity in rodent (compatible with the camera sensitivity) and the highest specific activity that must be reached to minimize the degree of receptor occupancy and achieve maximal specific binding of the radioligand (Lancelot and Zimmer, 2010).

Brain imaging typically identifies neurotransmitters at three locations in the neurotransmitter pathway: the presynaptic neuron, the postsynaptic neuron and the intraneuronal metabolism (Heiss and Herholz, 2006). For example, the radiolabeled cocaine derivative [11C]PE2I for the dopamine transporter estimates their density in pre-synaptic neuron membranes (Emond et al., 2008); the radiolabeled antagonist [11C]raclopride binds to D2 receptors, allowing their imaging at a postsynaptic level; and [18F]-DOPA, the radiolabeled precursor of dopamine, allows imaging of the dopamine metabolic pathway, essentially at a presynaptic intraneuronal level (Elsinga et al., 2006). Most examples derive from the dopaminergic system, but other neurotransmitter systems are increasingly being explored, such as the serotonergic, cholinergic and peptideergic systems. Since neurotransmitter dysfunction is hypothesized to contribute to numerous neuropsychiatric disorders, quantifying the density of post-synaptic receptors may help explain several diseases. According to the Molecular Imaging and Contrast Database (MICAD), there are currently more than 200 brain PET radiotracers developed at preclinical or clinical stages. Despite this large number of radiotracers, it has to be kept in mind that many receptor subfamilies, characterized by specific pharmacological ligands, do not have their own PET radiotracer yet.

Neurodegenerative processes

One of the most active domains in brain PET imaging is currently the field of neurodegenerative disorders, such as Parkinson’s and Alzheimer’s disease.

Parkinson’s disease

The loss of dopamine terminals in Parkinson’s disease can be detected in humans using [18F]DOPA, which is taken up and sequestered by dopaminergic nerve terminals. Alternatively, [11C]-labeled ligands label dopamine transporter sites (Ravina et al., 2005). These PET imaging approaches gained a new interest when cell transplantation strategies were used in therapeutic trials for Parkinson’s disease. During the first pilot studies in patients, the dopamine nerve terminals derived from transplanted cells were visualized using PET as described above (Brooks, 2003; Schumacher et al., 2000). In a similar manner in contemporaneous preclinical studies, cell transplants using either fetal midbrain (Brownell et al., 1998) or embryonic stem cells (Bjorklund et al., 2002) were studied using [18F]DOPA and microPET. These successful preclinical results were followed by similar clinical studies showing that [18F]DOPA changes reliably correlate with clinical outcome over the entire post-transplantation time course (Ma et al., 2010).

Alzheimer’s disease

Other important neurodegenerative diseases are Alzheimer’s disease and related dementias which will affect about 114 million by 2050 worldwide (Wimo et al., 2003). Because Aβ-amyloid plaques are the hallmark of Alzheimer’s disease pathology, much effort has gone into developing radiotracers that allow imaging of Aβ plaques in vivo. Among possible markers of Alzheimer’s disease, the Pittsburgh compound B ([11C]PiB) seemed to be a sensitive and specific marker of amyloid-deposition (Klunk et al., 2004), justifying numerous studies in patients (Kadir and Nordberg, 2010). However, the short physical half-life of 11C requires that a cyclotron be available on-site for production of the isotope, which prevents widespread clinical use. Consequently, three 18F-labeled tracers were developed and investigated in clinical trials as proprietary tracers for commercial distribution by pharmaceutical companies: flutemetamol, florbetapir, and florbetaben (Herholz and Ebmeier, 2011). Depending on approval by regulatory agencies, these radiotracers are expected to soon become clinically available and provide biomarkers to distinguish patients with Alzheimer’s disease from normal controls (but see Ewers et al., 2011) and those with other diseases that cause dementia. They might also be used as biomarkers to assess the effect of anti-amyloid therapy. Finally, alternative targets are currently in development for other neurodegenerative proteins: e.g., alpha-synuclein or tau protein (Fodero-Tavolletti et al., 2011; Kikuchi et al., 2010).

Neuroinflammatory processes

Neuroinflammation, which has to be distinguished from the classical inflammation response, is more and more closely associated with many neurologic disorders (e.g., stroke or trauma) when in acute and neurodegenerative diseases when chronic (Glass et al., 2010; Man et al., 2007). This explains that non-invasive imaging is increasingly applied for inflammation in the central nervous system to study its involvement in pathophysiology, to improve diagnosis and to develop and monitor therapies. Initially, [18F]FDG PET was used in several studies of brain inflammation since a high rate of glucose metabolism is found in active white blood cells. However, one should keep in mind that [18F]FDG consumption gives an estimate of tissue metabolic disturbance, but may not allow precise and specific quantification of an inflammatory process because, for example, of the high background of brain tissue.

A major feature of acute or chronic neuroinflammation is the activation of microglial cells (Graeber et al., 2011) accompanied by expression of the peripheral benzodiazepine receptor (PBR), also known as the 18 kDa-translocator protein (TSPO) (Venneti et al., 2006). Gene-expression studies in brains of animal models and humans have shown that PBR/TSPO expression rapidly increases on inflammation, making PBR/TSPO an attractive target for the imaging of brain inflammation. Radiolabeling of PBR ligands has enabled the imaging of PBR expression using PET, based on well-validated concepts and tools from the receptor neuroimaging field. About ten specific ligands have been successfully used for in vivo studies with human subjects (Schweitzer et al., 2010). [11C]PK11195 was the first tracer to be consistently used in PET imaging studies of neuroinflammation and, almost thirty years later, is still used in numerous studies (Chauveau et al., 2008). However numerous limitations of [11C]PK11195 have been pointed out: a high level of non-specific binding and a relatively poor signal-to-noise ratio, explaining why alternative PBR radioligands have been proposed.
Among these, $^{[1]}{\text{C}}$DAA1106, $^{[1]}{\text{C}}$BPR28 and $^{[18]}{\text{F}}$DPA-714 have demonstrated significantly increased binding in human brain (Scarff and Kassiou, 2011).

**Neurooncologic processes**

PET imaging in patients with brain tumors gives information on the metabolic state and molecular events within the tumor, complementary to MRI which reveals mostly anatomical information on the tumor. Neurooncology is a discipline between cancer science and neuroscience and its PET radiotracers mostly come from oncology. $^{[18]}{\text{F}}$FDG has been the tracer of choice for oncologic PET imaging, based on the increased glucose metabolism of most tumors. Despite its recognized limitations in brain tumor imaging due to the high background of normal gray matter, this imaging modality remains the most commonly used glioma tracer nowadays (Borbely et al., 2010). $^{[18]}{\text{F}}$FDG provides a global picture of the tumor, predicting aggressiveness, helping to differentiate recurrent tumor from treatment-related changes and discriminating pharmacosensitive tumors.

In recent years, the principal challengers of $^{[18]}{\text{F}}$FDG came from two major biochemical families: positron-labeled amino acid analogs, participating in the increased protein metabolism of glioma cells and nucleotide analogs, providing information on cellular proliferation activity. The major advantage of these radiotracers over $^{[18]}{\text{F}}$FDG is their lower background activity in normal brain tissue, which allows detection of low-grade tumors or small lesions. $^{[1]}{\text{C}}$methionine was one of the first radiolabeled amino acid analogs to be studied (Strauss and Conti, 1991). $^{[1]}{\text{C}}$methionine is probably the tracer of choice at this time, in terms of its sensitivity, specificity and accuracy, but its disadvantage is that $^{[1]}{\text{C}}$ has a short half-time. This disadvantage was eliminated by the development of $^{[18]}{\text{F}}$-labeled essential amino acid. In the late 1990s, $^{[18]}{\text{F}}$fluoroethyl-l-tyrosine was introduced in oncological research (Wester et al., 1999). Different tyrosine analogs have recently been developed with the hope of constituting detection of low-grade tumors or small lesions. $^{[18]}{\text{F}}$FDG provides a global picture of the tumor, predicting aggressiveness, helping to differentiate recurrent tumor from treatment-related changes and discriminating pharmacosensitive tumors.

Now the time has come to propose new applications for current radiopharmaceuticals in neuroimaging and to develop innovative probes for the renewal of PET neuroimaging in the field of molecular and personalized medicine. Although the past decades have seen identification of hundreds of molecular mechanisms and potential targets in the brain, there is still a bottleneck between the numerous molecules targeting receptors, transporters and enzymes and their corresponding PET probes. It is becoming easy to select a list of proteins that are relevant to any biologic mechanism from a literature search, but not every protein is useful for PET imaging, explaining the high attrition rate in the development of new PET radiopharmaceuticals for brain imaging. A good target has several characteristics: (i) it is expressed preferentially with higher levels in diseased than in normal tissue; (ii) it is highly expressed, although a low density can be detected thanks to the sensitivity of PET; (iii) it has to be accessible to the radiotracer, the first tangible barrier being the blood–brain barrier; and (iv) to increase the sensitivity and contrast, it is preferable that binding is constant during acquisition time (Reynolds and Kelly, 2011). The following examples will illustrate how these constraints can be managed.

**PET radiotracers for measuring endogenous neurotransmitter release**

Under certain circumstances, PET can provide a dynamic measure of neurotransmission by measuring acute fluctuations in synaptic neurotransmitter concentrations in the living brain. This function is based on the principle of competition between a particular radioligand and a neurotransmitter (Laruelle, 2000). Changes in receptor binding following a pharmacological intervention may indicate a modified number of available receptors or changes in receptor affinity. Several reports demonstrate that PET, using a ligand with a relatively low affinity for the receptor in question, might be able to evaluate the release of a neurotransmitter after a pharmacological manipulation. Initially, it was speculated that dopamine might compete with the ligand on the receptor, if the affinity of the ligand to its receptor is moderate (Seeman et al., 1989). For instance, $^{[1]}{\text{C}}$raclopride is a radiotracer with higher selectivity and more moderate affinity for dopamine $D_2$ receptors than dopamine (Cumming et al., 2002). PET has been applied to assess dopamine’s effects on striatal $^{[1]}{\text{C}}$raclopride binding to the $D_2$ receptor radiotracer in neurophysiology (Egerton et al., 2010), in psychiatry (particularly in addiction (Narendez and Martinez, 2008; Volkow et al., 2011) or schizophrenia (Abi-Dargham et al., 2004; Thompson et al., 2009)) and in neurology (particularly in Parkinson’s disease (Brooks, 2006; Sawamoto et al., 2008)). More recently, modifications in $^{[1]}{\text{H}}$MPPP binding (a serotonin $5-HT_3A$ receptor radiotracer) were reported in animal models, after pharmacologically-induced changes in extracellular serotonin (Aznavour and Zimmer, 2007). Despite the great interest of carrying out comparable studies in humans, clinical studies reported heterogeneous results, methodologically limited by the lack of direct measurement of brain serotonin as a control (Derry et al., 2005; Udo de Haes et al., 2012). Moreover, while the classical occupancy model predicts an association between changes in endogenous neurotransmitter levels and changes in the in vivo binding parameters of radiotracers, it does not fully explain the changes in radioligand binding in response to modulating neurotransmitter release (Ginovart, 2005; Laruelle, 2000). This explains that the search to a PET radiotracer designed to measure fluctuations in neurotransmitters is still open and controversial (Patersson et al., 2010). Agonist radiotracers, more sensitive to extracellular change in neurotransmitters, could be a perspective (see below).

**PET radiotracers for measuring the specific coupling of $G$ protein-coupled receptors with agonists**

G protein-coupled receptors, such as $5-HT_3A$, $D_2$ receptors and others, can exist in different states: a high-affinity state when coupled
with G proteins and a low-affinity uncoupled state (Emerit et al., 1990; Kobilka, 1992). Growing evidence suggests that G-protein receptor-coupling may be involved in both the pathogenesis and treatment of mood disorders (Schréiber and Avisar, 2007) or in neurodegenerative disorders (Thathiah and de Strooper, 2011). Therefore in vivo comparison of PET antagonist and agonist binding could provide a mean for "functional imaging" of neurotransmitter receptors.

The principle is that agonists preferentially bind to receptors that are coupled to G proteins, while antagonists are believed to label receptors indiscriminately (Mongeau et al., 1992; Nénonéné et al., 1994). Therefore, the comparison between a PET antagonist binding (thought to label a receptor family indiscriminately) and an agonist (thought to label G protein-coupled receptors of this family) could reflect the proportion of functional receptors. Further, agonist ligands may be more sensitive to extracellular levels of their neurotransmitter (Kegeles and Mann, 1997; Willeit et al., 2008). The neurotransmitter would bind preferentially to the high affinity state of the receptor and could therefore measurably displace a radiotracer antagonist from that state only. In contrast, an agonist radiotracer would be fully displaced upon increasing extracellular neurotransmitter.

Initial developments of cerebral PET agonists came from studies of dopamine receptors that described D2 receptor agonist PET radioligands in animal models (Cuming et al., 2003; Kortekaas et al., 2004; Tsukada et al., 2011). A few of these became radiopharmaceuticals and were compared to [11C]raclopride binding to quantify functional D2 receptors in patients (Finnema et al., 2010; Graff-Guerrerio et al., 2008; Willeit et al., 2008). Other developments came from 5-HT1A agonists developed as radiopharmaceuticals. Although current studies are still in animal models, they raise hopes for the future development of carbon-11- or fluorine-18 labeled 5-HT1A receptor PET radiopharmaceuticals (Kumar et al., 2007; Lemoine et al., 2010). The unusual binding profile of PET agonist could be related to regional differences in the coupling of receptors to G protein, opening the way to region-specific imaging of receptors and accompanying the emergent concept of "biased agonism pharmacology" (Newmann-Tancredi, 2011; Rebois et al., 2004).

**PET radiotracers for measuring receptor internalization**

Internalization of membrane receptors modulates their availability and is a key event in the regulation of neuronal functions: e.g., neurotransmitter release, desensitization/downregulation in physiological, pathological or therapeutic conditions (Bernard et al., 2006). The in vivo detection of this phenomenon in humans by PET is promising in terms of clinical management, particularly as an early biomarker of pathological or therapeutic conditions (Bernard et al., 2006). The in vivo comparison of PET antagonist and agonist binding could provide a mean for "functional imaging" of neurotransmitter receptors.

According to Laruelle (2000), changes in radioligand binding consequent to the modulation of neurotransmitter release are not fully accounted for by the occupancy model. Receptor trafficking could play an important role in transmitter–radioligand interaction in vivo, particularly in the case of D2 receptors or 5-HT1A receptors. In the case of [11C]FIMPFP, receptor internalization may be particularly significant in terms of receptor–ligand interactions. As documented in the rat, there is a rapid but transient internalization of 5-HT1A auto-receptors after acute administration of a direct or indirect agonist (Riad et al., 2001, 2004). In parallel experiments it was demonstrated that this internalization is associated with a considerable decrease in [11C]FIMPFP binding in rats (Riad et al., 2004; Zimmer et al., 2004) or in anesthetized cats (Aznavour et al., 2006). It was soon realized that PET imaging with a suitable radiotracer might provide a means to detect receptor internalization in human brain and possibly assess pharmacological responsiveness. A preliminary study in healthy subjects recommended [11C]FIMPFP imaging of the 5-HT1A auto-receptor internalization (Siben et al., 2008). However, the possible reasons for the sensitivity of a specific radiotracer to internalized receptors remain to be investigated. The relatively low lipophilicity or lesser affinity of the radiotracer for internalized receptors in a low sodium environment, and/or a conformational change in internalized receptors with occlusion of the radiotracer binding site, need to be considered. Although this approach is still at a proof-of-concept stage and presents methodological limitations, it opens a new use for several PET radiotracers in brain imaging.

**PET radiotracers for measuring P-glycoprotein function**

P-glycoprotein (P-gp) is an efflux transporter controlling the pharmacokinetics and the brain bioavailability of various compounds (Fromm, 2004). P-gp-mediated drug efflux was initially described in the field of oncology, and was suggested as playing a role in multidrug-resistant cancer. More recently, P-gp has been studied in various brain pathologies, e.g., medication-refractory epilepsy, Parkinson’s disease and Alzheimer’s disease (Lee and Bendayan, 2004). In PET imaging, the first studies concerning P-gp highliganded that many PET radiotracers are substrates for this brain efflux transporter, which disturbs their brain entry (Pike, 2009). This efflux transporter behavior of some radiotracers raised the possibility of studying efflux transporter function in vivo with PET. To enhance the understanding of its in vivo function under pathophysiological conditions, substrates of P-gp were radiolabeled and imaged using PET. One strategy to quantify brain P-gp function is to radiolabel a substrate (rather than an inhibitor) selective for P-gp. The difference in uptake in the target tissue before and after inhibition of the transporter will reflect P-gp function. To ensure that P-gp function is measured accurately, substrate radioligands must have high selectivity for P-gp, must produce a large signal after P-gp blockade and must generate few radiometabolites that enter the brain (Kannan et al., 2009). The most widely studied radiotracer is [11C]verapamil either as racemate or single R-enantiomer (Ikoma et al., 2006). However the radioactive metabolites of this radiotracer complicate its quantification with measurements of arterial inputs. This raises a question as to the practical utility of [11C]verapamil for quantitative measurement of P-gp function. Alternatively, [11C]dLop, a N-methyl-11C-labeled metabolite of the anti-diarrheal opiate, loperamide, is a promising radiotracer of P-gp function since it seems devoid of lipophilic radiometabolites (Kreis et al., 2010; Lazarova et al., 2008).

**PET radiotracers for measuring biomarkers during drug development**

The use of imaging biomarkers for the development of drug therapies, a field termed "pharmaco-imaging", has become more common in recent years. Several characteristics of PET biomarkers favor their use in preclinical and clinical pharmacological tests by: (i) validating the binding drug localization; (ii) establishing the transport efficiency of a drug to the target tissue in the brain thanks to PET microdosing, consisting in direct radiolabeling of drug molecules to study their tissue distribution and pharmacokinetics; (iii) establishing the drug occupancy of the saturable receptor sites; and (iv) determining the half-time occupancy of the drug (Wagner and Langer, 2011). This is particularly advantageous when compared with the classical pharmacological approach because of the opportunity to explore brain neurochemistry in vivo and because of the decreased duration of experiments, thanks to longitudinal studies.

Consequently, there is increasing evidence that PET imaging can accelerate the drug development process by revealing information early regarding drug effectiveness and safety for both research and regulatory purposes (Pien et al., 2005; van Gool et al., 2010). This implies that the candidate-drug must be developed in parallel with its PET radiotracer by combining molecular therapeutics and molecular diagnostics (Cross and Cruciani, 2010; van Gool et al., 2010). PET imaging can then be used in each phase of drug discovery and development, particularly in the later phases of preclinical screening when candidates are less than ten in number. This is important because it is difficult to simultaneously validate radiolabeling in a greater number of drug candidates.
The desired design properties of molecular imaging probes and drugs are similar in terms of affinity, specificity, blood–brain barrier penetration, and low rates of metabolism, although there are differences in terms of plasma clearance: short times are preferred for radiotracers (minutes to hours) and longer times for drugs (hours to days). After preliminary rodent studies, initial patient trials are performed with the molecular imaging probes and labeled drugs to assess how the animal findings compare to those in patients.

Although the largest pharmaceutical companies have bought or developed their own preclinical PET centers, other pharmaceutical companies are beginning now to outsource biomarker activities. These external partners may be platform providers, academic technology centers or diagnostic companies that execute well-defined biomarker discovery or development activities as needed to support the pharmaceutical project. Finally, for larger projects directed at developing new molecular imaging probes or drug development, participants with complementary expertise will need to cooperate. Partners may include pharmaceutical firms, biotech companies, diagnostic companies and academic laboratories or governmental agencies united by national or international funding programs, such as EU-PET in Europe or PhRMA in the USA.

**PET radiotracers for measuring in a multiduality manner?**

**Instrumental multimodality**

The information content of PET images only provides anatomical landmarks incidentally. PET brain images obtained using extremely specific molecular probes that target only certain populations of neurons are difficult to interpret on their own. Therefore, combining two or more anatomical imaging modalities can provide complementary information. Although the combination of PET and computed tomography (CT) has already occurred in clinical and preclinical scanners, PET–CT has many limitations in neuroscience research since CT has limited soft-tissue contrast and is therefore not suitable for brain imaging. The combination of PET and Magnetic Resonance Imaging (MRI), which has excellent soft-tissue contrast, particularly for cerebral tissue, as well as a capability for MRI (functional Magnetic Resonance Imaging), MRS (Magnetic Resonance Spectroscopy) and perfusion measurements is therefore promising. The combination of PET and MRI has many potential applications for investigating neurotransmitters, receptor density or metabolite concentration. By contrast, two stand-alone systems, allowing only sequential data acquisition and subsequent image fusion, cannot provide such powerful information. The first prototypes developed combined PET/MRI scanners for small animals, based on a 7 Tesla animal MRI scanner (Catana et al., 2006; Judenhofer et al., 2007, 2008; Pichler et al., 2006). Clinical PET/MRI scanners were recently developed according to the same instrumental architecture and the first studies in neurology have now been published (Boss et al., 2010; Pichler et al., 2010).

**Tracer multimodality, a myth?**

It has to be recognized that in the field of imaging, “multimodal tracer” are frequently used words, not to say a “fashion”. The main interest for multimodality imaging is to use coregistration to enhance image interpretation or quantification, (i.e. PET and MRI), thanks to the previously described multimodal cameras. However, physical and pharmacological rules make this concept unrealizable. On one hand, MRI requires tens to hundreds of micromolar of agent to modify the relaxivity of surrounding water molecules, (e.g., gadolinium chelates), and molar equivalent of each modality. In other words, a molecule with an equimolar concentration of effector molecule, would require a final concentration equal to the least sensitive imaging modality (MRI), leading to excessive radioactivity or insufficient specific activity, violating the tracer principle of PET. This confirms that PET brain tracers still have their own place and specificity in the dynamic field of molecular imaging.

**Conclusions**

Brain molecular imaging is becoming a reality thanks to PET radiotracers enabling non-invasive measurement of receptor numbers, receptor binding affinity, metabolic rates of physiological pathways, and concentrations of molecular end-products, in addition to other normal and disease-specific signatures of molecular activity. At this time, numerous brain receptors, transporters, extracellular enzymes and intracellular macromolecules are potential targets for PET molecular imaging. We highlighted some approaches that are in development, as well as some areas where more progress is needed. The ultimate goal of brain PET radiotracers is the non-invasive localization and quantification of proteic function (e.g., internalization, G-protein coupling, active transport etc.), and profiling of signal transduction pathways, (i) to get further insight into the molecular pathophysiology of brain diseases; (ii) to facilitate the design of diagnostic biomarkers; and (iii) to speed up the development of new therapeutics. Development of brain radiopharmaceuticals is an exciting and rapidly evolving field, implying interdisciplinary research bringing together individuals from a wide range of disciplines, including neuropharmacology, biochemistry, chemistry and imaging science.

**References**


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