Neuroimaging in Psychiatric Drug Development and Radioligand Development for New Targets

1

Akihiro Takano, Christer Halldin, and Lars Farde

Contents

Abstract

Positron emission tomography (PET) is an imaging modality used to measure physiological and biochemical markers in brain. Neuroreceptors, transporters, or enzymes are visualized and quantified with appropriate PET radioligands. In the development of drugs for treatment of psychiatric disorders, there are three major applications of PET. First, PET microdosing is used for pharmacokinetic evaluation. By injection of minute amount of radiolabeld drug, information about brain exposure can be obtained already at the early phase of drug development. Another application is receptor occupancy studies. Here, the competition between a drug and a PET radioligand binding is examined at the target sites. The competitive effect is useful to have when selecting the doses tested in further clinical trials. The third application is to use imaging biomarkers for diagnosis or efficacy. To widen the use of PET, the development of the PET radioligands for new targets is vital. Several criteria and characteristics such as binding affinity, selectivity and lipophilicity are important when selecting new PET radioligand candidates for targets in brain.

A. Takano (✉) • C. Halldin • L. Farde
Department of Clinical Neuroscience, Center for Psychiatric Research, Karolinska University Hospital, R5:02, Stockholm SE-171 76, Sweden
e-mail: akihiro.takano@ki.se

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1.1 Introduction

Drug development requires considerable investments of time and money. Since the technique of binding assay was introduced in the late 1950s (Yalow and Berson 1959), numerous compounds have been selected based on in vitro affinity data, evaluated in preclinical models and subsequently tested for efficacy in psychiatric diseases such as schizophrenia and mood disorder. However, as the pathophysiology of psychiatric diseases has not been fully understood, the industrial drug projects have had an evident element of "trial and error." Lack of or insufficient efficacy is thus a major reason for attrition and adds to failure for safety reasons (Arrowsmith 2011a, b). In some drug projects, the failure may be related to difficulties with dose finding. In other words, the doses used in preclinical and clinical trials were too low or too high. The fundamental question is thus whether the drug failed due to suboptimal brain exposure and target engagement of the drug or whether the target was invalid.

Positron emission tomography (PET) is an imaging modality by which it is possible to measure physiological and biochemical markers in the brain by using appropriate radioligands. Most PET radioligands are labeled with radionuclides having a short half-life such as C-11 (half-life, 20.4 min) or F-18 (109.8 min). Following the successful introduction of PET for neuroreceptor imaging in the 1980s (Farde et al. 1986), the technique has been widely used to visualize and quantify drug target sites, mainly neuroreceptors, enzymes, and transporters in the human brain in vivo.

In this chapter, we will focus on the major applications of PET in drug discovery and development. The need for development of novel radioligands for new targets will be given particular attention.

1.2 PET Application for Drug Development

1.2.1 PET Microdosing for Pharmacokinetic Evaluation

After radiolabeling of the drug itself with short-lived radionuclides, such as C-11 or F-18, the distribution of the drug can be examined in the living body. This approach has been referred to as "microdosing" (Lappin and Garner 2003). A "microdose" is defined as a dosage level less than 1/100 of the dose estimated to induce a pharmacological effect. In addition, a maximum dose has been set to 100 μg (EMEA 2003). Due to effective radiosynthesis and high specific radioactivity, the dose administered in a PET study is usually less than 1 μg.

There are several other approaches for microdosing, such as accelerator mass spectrometry (AMS) and LC/MS/MS. AMS is an ultrasensitive methodology that can be used to quantify C-14 in biological samples such as blood, urine, or tissue biopsies. LC/MS/MS is a technique that can measure very low

concentrations of unlabeled compounds in plasma, urine, or CSF. When compared with AMS and LC/MS/MS, PET has the advantage of extending the microdose concept from body fluids to organs in the whole body. In other words, AMS and LC/MS/MS technologies provide pharmacokinetic information based on the plasma levels of the compounds. PET extends traditional pharmacokinetics by providing information about drug concentration in the target organ or region. However, a limitation is that the short half-life of the PET radionuclides limits the time of data acquisition to about 2 h for C-11-labeled drugs and 12 h for F-18-labeled drugs.

Some failures in CNS drug development have been attributed to poor brain exposure of the drug (Taylor 2002). The PET-microdosing approach may thus be of particular importance in the development of CNS drugs since it has the potential to confirm sufficient brain exposure in early phase of drug development. The information is of particular value before investments are made into expensive phase II and III trials.

At least 1 % of injected radioactivity in the brain has been the finding for most of the drugs viewed as having acceptable brain exposure. However, no strict guidelines have yet been established for decision making. To efficiently translate small animal's results into human condition, microdosing PET study of nonhuman primate (NHP) is a useful intermediate since it can serve as a good predictor of brain exposure of the candidate drugs (Schou et al. 2013).

Although the drugs used in the field of psychiatry are mainly targeted to the CNS, whole body PET measurements can provide useful information in relation to potential side effects. A limitation of PET is that the radiolabeled drug will be metabolized in the living body. Measurement of radioactivity by PET machine can thus not differentiate the parent radiolabeled drug from radiolabeled metabolites. To overcome this problem, radiolabeling and administration of the metabolite only may provide additional information (Seneca et al. 2009).

Although the requirements for preclinical safety data for microdosing study have been reduced by regulatory authorities (Verbruggen et al. 2008), the radioligand production has to follow good manufacturing practice (GMP) (US FDA Code of Federal Regulations Title 21). This requirement has increased the costs of PET-microdosing studies in the human subjects.

1.2.2 PET Receptor Occupancy to Demonstrate Target Engagement and in Relation to Pharmacodynamics

A number of PET radioligands have been developed for several key targets related to neurotransmission (Table 1.1 and Fig. 1.1) (Halldin et al. 2001). Using these tools, it is possible to map and quantify the in vivo distribution of the target neuroreceptors or transporters. Details on quantification of the radioligand binding are described elsewhere in this textbook (Chapter 2).

Table 1.1 Representative PET radioligands for neurotransmitter systems

Neurotransmitter system		PET radioligand
Dopamine	D1	[^{11}C]SCH23390
		[^{11}C]NNC112
	D2	[^{11}C]raclopride
		[^{11}C]FLB457
	Transporter	[^{11}C]PE2I
		[^{18}F]FEPE2I
5HT	1A	[^{11}C]WAY10065
	1B	[^{11}C]AZ10419369
	2A	[^{11}C]MDL100907
	Transporter	[^{11}C]MADAM
		[^{11}C]DASB
GABA-benzodiazepine		[^{11}C]Flumazenil
		[^{18}F]Flumazenil
		[^{11}C]Ro15-4513
Norepinephrine	Transporter	[^{18}F]FMeNER-D2
Cannabinoid	CB1	[^{11}C]MePPEP
		[^{18}F]FMPEP-d2

The change of radioligand binding between baseline and after drug administration is used to calculate the drug occupancy at the target neuroreceptor, transporter, or enzyme (Figs. 1.2 and 1.3).

PET determination of receptor occupancy has been most extensively applied for antipsychotic drug binding to the dopamine D2 receptor (Farde et al. 1988). The relationship between in vivo dopamine D2 receptor occupancy and antipsychotic drug effect was early established. More than 65–70 % of dopamine D2 receptor occupancy is required to obtain antipsychotic efficacy, but at more than 80 % of occupancy, there is a high risk for extrapyramidal symptoms (Farde et al. 1986; Kapur et al. 2000). The atypical antipsychotic clozapine is an exception since this drug has antipsychotic effect at lower dopamine D2 occupancy (Farde et al. 1992; Nordström et al. 1995).

The PET occupancy approach has now become widely applied to drug development and extended to several other targets including the serotonin and noradrenaline neurotransmission systems and enzymes such as monoamine oxidase B (Meyer et al. 2004; Hirvonen et al. 2009; Sekine et al. 2010). The target occupancy by a new candidate drug is usually estimated for different doses, so that the curvilinear relationship between dose/plasma level and occupancy can be established (Fig. 1.4). This key information will help efficient dose setting in phase II and III studies by avoiding doses that are too low or too high. For new targets, the relationship between target occupancy and clinical efficacy or side effects may be insufficiently understood. In such cases, the relationship between occupancy and pharmacodynamics can only be established after phase II and III studies when clinical data becomes available.

A recent successful example of the occupancy approach is [^{11}C]AZ10419369, a PET radioligand for the serotonin 5HT1B receptor subtype (Figs. 1.2, 1.3 and 1.4). This radioligand was developed in a collaboration between Karolinska Institutet and AstraZeneca and has been used for the occupancy measurement by AZD3783,

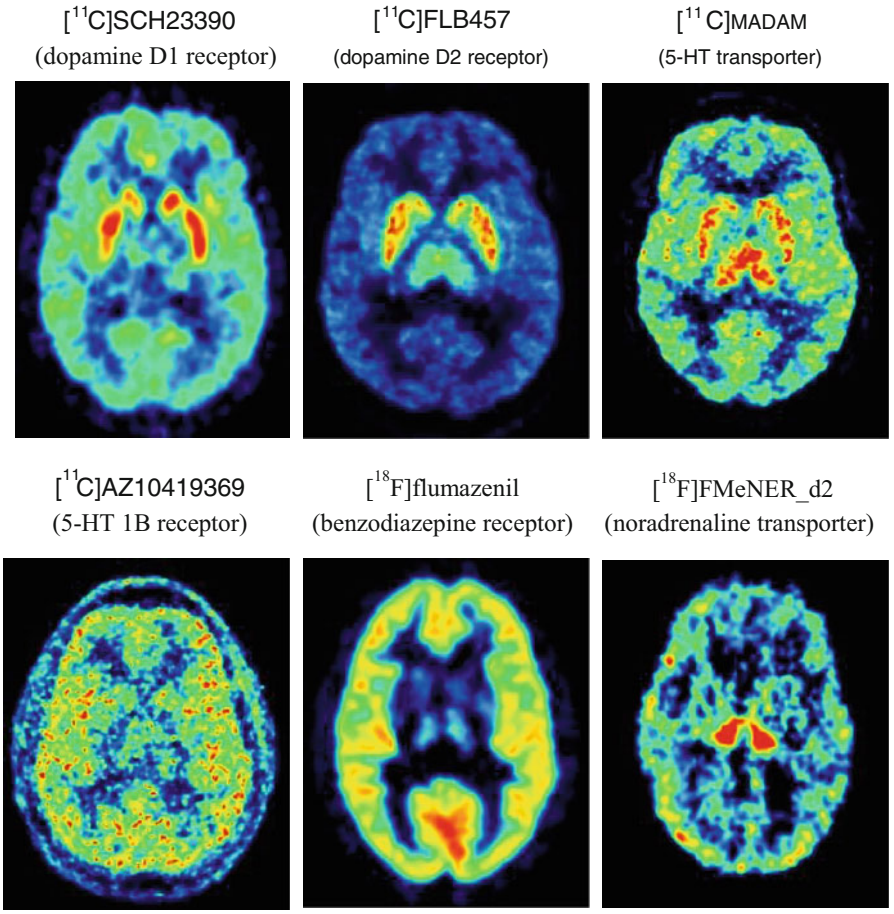

Fig. 1.1 Horizontal brain sections through the striatal level showing the regional distribution of the binding of commonly used PET radioligands. Images represent radioactivity summed after radioligand injection (9–51 min for [11C]SCH23390, 0–87 min for [11C]FLB457, 7–93 min for [11C]MADAM, 3–63 min for [11C]AZ10419369, 9–93 min for [18F]flumazenil, 90–210 min for [18F]FMeNER_d2)

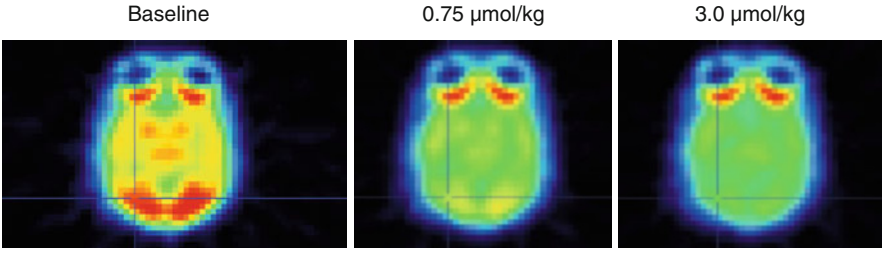

Fig. 1.2 Horizontal PET images showing [11C]AZ10419369 binding to the 5HT1b-receptor at baseline and after AZD3483 i.v. administration in a cynomolgus monkey

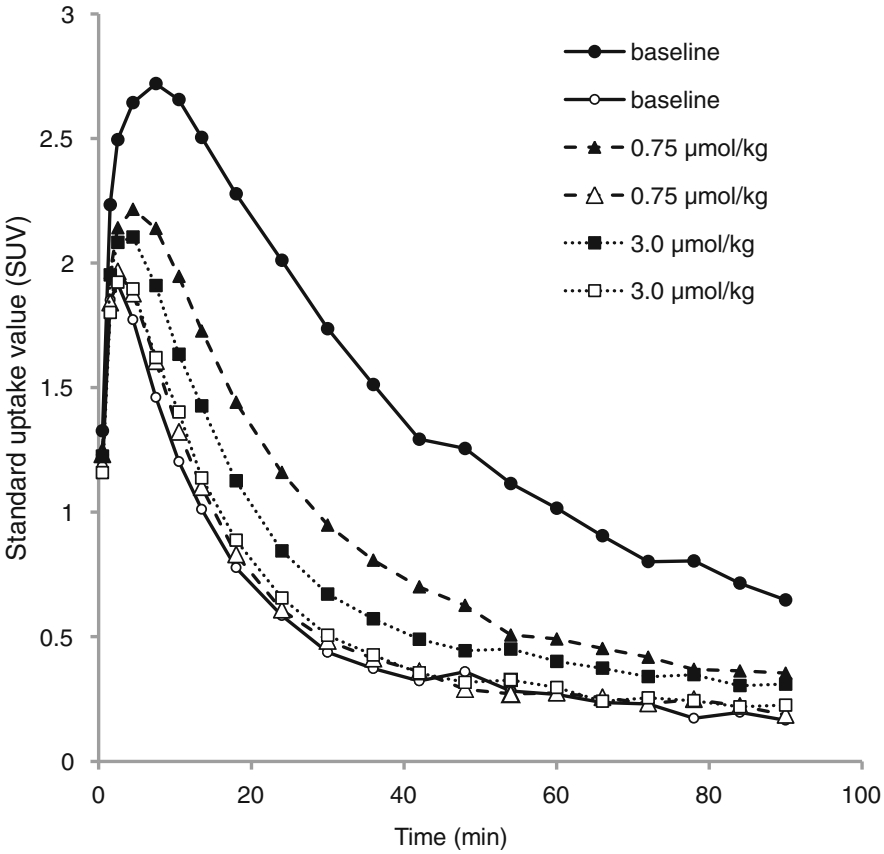

Fig. 1.3 Time activity curves for the regional [^{11}C]AZ10419369 binding in a cynomolgus monkey illustrated in Fig. 1.2. *Filled marks* represent occipital cortex. *Open marks* represent the cerebellum

a candidate drug for treatment of depression (Pierson et al. 2008; Varnäs et al. 2011). The occupancy estimations were first performed in NHP and later in human subjects. The relationship between the dose and 5HT1B occupancy by AZD3783 was similar between nonhuman primates and human subjects (Varnäs et al. 2011). Despite the value demonstrated for nonhuman primate studies of AZD3783 to predict binding in the human brain, some caution must be exercised whenever making such predictions for new drug targets.

In an optimal occupancy study, a wide range of doses are investigated, ideally covering the interval from 0 to 100 %. However, in reality, due to the risk of side effects, the selection of the doses administered to human subjects is likely to be limited to lower doses. Due to a limited range of data, it may thus be difficult to confirm whether a maximal occupancy can be reached and whether the binding affinity estimates (Kiplasma values (Karlsson et al. 1995)) are reliable.

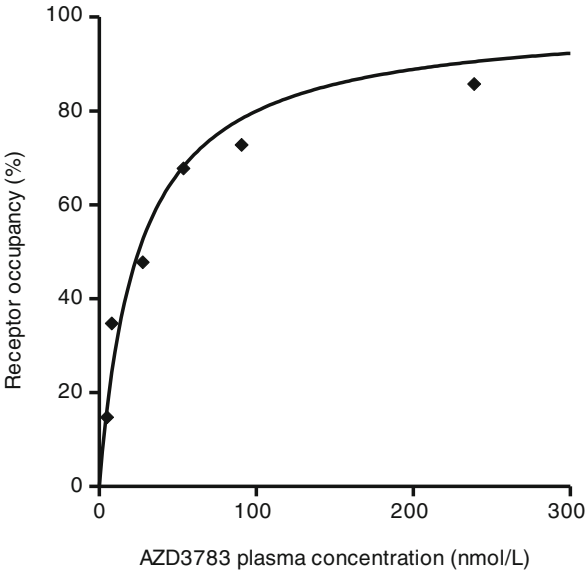

Fig. 1.4 The curvilinear relationship between plasma concentration of the 5HT1b receptor antagonist AZD3783 and target receptor occupancy in cynomolgus monkeys (Modification of figure in Varnäs et al. 2011)

For some drugs, a pharmacologically active metabolite having affinity for the target may contribute to occupancy at the target sites (Takano et al. 2011). In NHP, the occupancy of the metabolite can be estimated by injection of the metabolite only. PET in NHP may thus provide additional useful information prior to the human PET study.

1.2.3 Pathophysiology Biomarkers for Diagnosis or Efficacy Studies

For most psychiatric disorders, there are not generally accepted biomarkers in spite of considerable efforts to reveal the pathophysiologies. The recent progress in neuroimaging of psychiatric disorders will be discussed in detail in other sections of this textbook.

A general approach applied in drug development is to use PET to measure physiological parameters such as cerebral blood flow or brain glucose metabolism using [^{15}O]H_2O or [^{18}F]FDG. Change in cerebral blood flow or brain glucose metabolism at drug treatment can thereby be detected, which indirectly serves to confirm a drug effect in the brain. The combined study of occupancy at a biochemical marker and a physiological biomarker has a promising potential to further confirm target engagement but has so far been utilized in a few studies only (Halldin et al. 2001).

In a back-translational approach, animal models for psychiatric disorders can be investigated using micro-PET (Higuchi et al. 2010; Klunk et al. 2004). As the animal does not have to be sacrificed after each PET measurement, longitudinal evaluation of chronic administration of the candidate drugs can be performed. Such

translational approaches have potential to validate animal models in relation to the pathophysiology and clinical treatment of psychiatric disorders.

In the field of neurology, amyloid imaging in Alzheimer disease (AD) has been successful (Klunk et al. 2004; Rinne et al. 2010; Jack et al. 2011; Cselényi et al. 2012; Gelosa and Brooks 2012; Mathis et al. 2012). Building on the historical observation of amyloid deposits in AD brains postmortem, the reference radioligand [^{11}C]PIB was developed to allow for in vivo imaging (Klunk et al. 2004). Recently, numerous new and improved PET radioligands for amyloid imaging have been reported such as [^{18}F]AZD4694 (Cselényi et al. 2012; Gelosa and Brooks 2012; Mathis et al. 2012). The PET radioligands have initiated a series of studies on the pathophysiology and clinical diagnosis of AD (Jack et al. 2011). In addition, PET imaging of amyloid deposits in AD has shown potential to detect effects of drug treatments aimed at reducing the amyloid plaque burden (Rinne et al. 2010).

There is however no established biomarker for the diagnosis of psychiatric disorders based on pathological evaluation postmortem. Despite that, there might be some potential to develop imaging biomarker. Recently imaging of the translocator protein (TSPO), which indicates activated microglia activity, has shown higher binding in schizophrenia (van Berckel et al. 2008; Doorduin et al. 2009) as well as correlation between TSPO binding and clinical symptoms of schizophrenia (Takano et al. 2010). Such development of new imaging markers could also be useful as efficacy biomarker in psychiatric drug development.

As discussed above, the discussed PET approaches can provide unique information to facilitate drug development. However, the success of the PET study depends on the development of appropriate PET radioligands. As shown in Table 1.1, the availability of PET radioligands is not yet sufficient. As the list of candidates for the drug development has expanded diversely, the need for novel PET radioligand development for new targets becomes critical.

1.3 Radioligand Development: Targeting Neurology and Psychiatry

The selection of radioligands for PET is initially guided by data obtained in vitro by using a tritiated radioligand or by displacing a reference radioligand with the unlabeled molecule. In vitro binding normally provides information regarding ligand *affinity* (e.g., the dissociation equilibrium constants K_d or K_i) and *selectivity* (i.e., the relative affinity to competing binding sites) as well as regarding the *concentration* of binding sites (B_{max}). The optimum affinity is closely related to the expected B_{max}. It is preferable if the B_{max} clearly exceeds the K_d of a ligand, i.e., if a binding site exists in vivo at nanomolar concentrations, a potentially successful radioligand ideally should have a subnanomolar affinity. Binding affinity is an important factor that determines the *ratio* of specific binding to nonspecific binding. The higher the ratio, the more sensitive the signal is likely to be to changes in available binding site concentration, caused by disease or drug occupancy.

Binding affinity (i.e., the fraction of dissociation rate constant, k_{off}, and association rate constant, k_{on}) usually governs the approach to be taken in the biomathematical modeling of the ligand-receptor interaction. If the binding of a radioligand is reversible over the time scale of a PET experiment (i.e., a "transient equilibrium" is attained), *equilibrium* approaches toward quantification can be utilized. On the contrary, irreversible ligands normally demand for *kinetic* modeling, wherein the transfer of radioligand between pharmacological compartments is described in terms of rate constants. This approach requires in most cases the determination of an *input function* (i.e., the time course of free radioligand in plasma), which makes the measurement of radiometabolites in arterial plasma necessary by radio-HPLC. Very high binding affinity of a radioligand in combination with a comparatively slow clearance from tissue can restrict its usefulness for PET, as the rate-limiting step of tracer retention may become the delivery – instead of the binding process.

A further important criterion for a radioligand is binding selectivity. Ideally, the affinity of a radioligand should be greatest for the site of interest by one order of magnitude. Lack of selectivity may be acceptable if nontarget sites are separated anatomically from the target binding sites. Most neurotransmitter receptors have now been found to exhibit multiple subtypes, and radioligands that were initially thought to bind to a single class of receptors truly display affinity toward several subtypes. Most benzamides are equipotent at the dopamine D_2 and D_3.

Another substantial consideration in the development of a new radioligand is estimation of *nonspecific binding*. This is an essentially non-saturable component of the total tissue uptake of a radioligand, usually attributed to adhesion to proteins and lipids. Nonspecific binding and its clearance in vivo are difficult to predict absolutely. Within a class of structurally related compounds, nonspecific interactions with tissue generally increase with increased lipophilicity. The logarithm of the partition coefficient between water and octanol (log *P*) is often taken as a useful index for the lipophilicity of a compound in the context of biological systems. But some degree of lipid solubility is needed for good passage over the blood–brain barrier, which is a prerequisite for satisfactory counting statistics. However, the lipophilic nature of a molecule might also favor binding to plasma proteins, thus reducing the available "free fraction" in blood that is capable of diffusing through membranes. Taken together, it appears that there is an optimal – but rather narrow – "window" of lipophilicity for brain radioligands, which optimally should be 1.5–2.5.

PET measures the regional radioactivity concentration without being able to distinguish the chemical forms or environments in which the radioactivity resides. For a clearly interpretable signal, it is therefore necessary that radiometabolites do not contribute to specific binding. Thus, radioligands should be preferably resistant to rapid metabolism over the period of data acquisition. Furthermore, radiometabolites should not be taken up in the target area. This requirement may have important consequences concerning the elaboration of a radiolabeling strategy. In fact, the position of the radiolabel within a molecule might be crucial for the in vivo usefulness of a radioligand, as the major drawback for not useful radioligands is radiometabolites also penetrating the blood–brain barrier (BBB).

An important factor is the specific radioactivity (SA) of the radioligand. Too low SA may result in pharmacological side effects or toxicity of the radioligand. Moreover, low SA may saturate the biological system of interest, thus no tracer condition. For low-density binding sites, very high SA is essential in order to exclude a substantial occupation of target sites by unlabeled compound.

It has to be emphasized that any data extracted from in vitro experiments can only give a rough estimate of the situation to be encountered in vivo. Most in vitro assays use homogenized tissue, which does not reflect the tissue heterogeneity in the intact organ in vivo. Competition with endogenous ligands may lower the binding of a radioligand at a given site. It has to be kept in mind that neurotransmission systems in the intact body constitute part of a dynamic and communicating environment and that neural interaction may actually alter in vivo receptor binding.

If a potential candidate radioligand has been identified and a labeling technique developed, some preclinical evaluation, prior to PET in humans, needs to be performed. Some useful information may be obtained by studies in rodents. Typically, the radioligand is injected intravenously into a series of rats or mice. These are then sacrificed at known times after injection and different organs are removed and counted, thus providing the distribution of the radiotracer in different organs over time. In addition to that, clearance of radioactivity from plasma and information on the appearance of plasma radiometabolites can be obtained. Specificity of binding may be demonstrated by using selective and potent unlabeled agents that act in a competing way at the site of interest. Small-animal PET devices are becoming increasingly available which promise to simplify radioligand evaluation. It should be noted, however, that species differences may be encountered and lead to different results between animals and human subjects.

A complementary tool in early radioligand development may be *autoradiography* experiments, wherein frozen slices of tissue obtained from the organ of interest such as the brain are mounted on glass slides and incubated for a given time with a buffered radioligand solution. These sections are exposed to radiation-sensitive film or preferable using a phosphor imager. Autoradiography may provide information if a radioligand is suitable for PET, especially regarding affinity, selectivity, and nonspecific binding. An advantage compared to in vitro homogenate binding assays is the use of intact tissue, which provides information in an anatomical manner. But, no data regarding the in vivo pharmacokinetics of the radioligand can be deduced from such an experiment. This lack of information can be compensated for by ex vivo autoradiography approaches, where the analysis is done in vitro after in vivo administration of the radioligand into small animals. Usually, autoradiography experiments are performed with ^{3}H-labeled compounds, but also molecules labeled with positron emitters can be used, but this lowers the spatial resolution. A superior method is using whole hemisphere autoradiography of the *postmortem* human brain.

The next step in radioligand development is normally PET in nonhuman primates – such as cynomolgus monkey. Analysis of plasma radiometabolites from venous blood samples provides useful information regarding clearance and metabolic pathways. Administration of potent and selective competing agents prior to

radioligand injection (*pretreatment*) or during the time course of the PET experiment (*displacement*) can demonstrate the specificity and reversibility of radioligand binding.

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