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4.1 Introduction

Radiotracers and radiopharmaceuticals for Nuclear Medicine imaging with applications in neurology have been implemented for more than 50 years, based on the research efforts of scientists in the academic world, hospitals, and industry. The first described imaging applications in humans were pioneered in the early 1950s in the USA for the detection of intracranial lesions and for the assessment of regional cerebral blood flow. Images were obtained using conventional gamma cameras, after administering inert radioactive gases by inhalation or radioactive metal complexes by injection. These agents were not aimed to detect any specific mechanism or molecular target, but they were used in the research setting for describing pathophysiological processes.

The strong efforts in radiochemistry, in addition to the technological improvements of the tomographic detection systems, contributed to enforce the interest for this emerging technique,

and the first studies in a clinical setting were reported during the 1960–1970s. The potential impact to patient care boosted the need of new and more specific radiotracers. Thus, the development of new tracers for neuroimaging applications turned from using radioactive salts and known chelating agents to the modern concept of molecular neuroimaging. This latter model is based on the specific interaction between the imaging agent and the molecular structure or cellular process of interest, such as receptors, enzymes, transporters, or metabolic pathways. The emitting radionuclides contained within the agent provide a signal that can create an image if associated with the proper imaging system. After four decades of hard work in radiochemistry, hundreds of radiotracers containing different single photon and positron emitter nuclides have been developed and applied to the most common imaging modalities for the evaluation of brain function, accomplishing clinical and investigational requirements (Table 4.1). A number of them have been approved by authorities and are currently used in Nuclear Medicine departments by physicians for specific applications, showing a potential impact for direct patient care. The major clinical areas of application include cerebrovascular diseases, movement disorders, dementia, epilepsy, and brain tumors. Neuroimaging radiotracers are also employed for the study of other mental disorders such as schizophrenia, addiction, depression, and anxiety.

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Table 4.1 Early PET and SPECT human brain studies

Date	PET	SPECT
1955		[⁸⁵ Kr]Gas
1963		[¹³³ Xe]Gas
1964		[^{99m} TcO ₄ ⁻
1965	[⁶⁸ Ga]EDTA	
1967	[¹⁵ O]Oxygen	
1969	[¹⁵ O]Water	
1975		[¹²³ I]Iodoantipyrine
1976	[¹¹ C]Methionine	
1977	[¹⁸ F]FDG [¹¹ C]Tryptophan [¹¹ C]Valine	
1981		N-isopropyl-[¹²³ I] Iodoamphetamine ([¹²³ I]IMP)
1983	N-[¹¹ C]Methylspiperone [¹⁸ F]FDOPA	[¹²³ I]IQNB
1984	[¹⁸ F]Cyclofoxy	
1985	[¹¹ C]Raclopride [¹¹ C]Carfentanil [¹¹ C]Flumazenil	[^{99m} Tc]HMPAO
>1985	Many	Many

Unexpectedly, the expanded range of radiotracers hasn't been followed by a parallel introduction of radiopharmaceuticals, as tools for answering clinical needs. During the last decade, radiotracers in order to get approval for clinical use or marketing authorization have been facing implemented radiopharmaceutical requirements, similar to those that are broadly applied to all drugs. In particular, the increased awareness for the quality and safety of radiopharmaceuticals and the need for confirmation that the diagnostic agent has to provide clinically useful information contributed to make a widespread use of new agents very challenging.

Last but not least, the use of SPECT and PET neuroimaging is not restricted to the clinical or investigational field in the hospital Nuclear Medicine departments. During the last decades, this imaging technique has gained attention in neuroscience for the excellent capability to gain insight into disease mechanisms, describing the kinetics of the radiotracers and measuring target reserves, such as enzymes or receptors. For this reason, the standard methodology developed to study normal and pathological processes has been extended to support drug discovery in the

universities and in the pharmaceutical industry. Neuroimaging with carbon-11 and fluorine-18 PET radiotracers has become a routine procedure for the development of biomarkers for novel central nervous system therapeutics. Using a PET-specific radiotracer identical to a drug candidate, brain penetration and target occupancy measurements can be defined. The drug interaction with a specific brain target can also be studied by competition, if a suitable radiopharmaceutical for the same target is available. Such information may have a substantial impact in a go/no-go decision on a set of drug candidates before first-in human studies and furthermore may produce a significant effect on the overall pipeline cost for drug development.

4.2 Brain SPECT and PET Radiotracers: A Journey More than 50 Years Long

From a historical perspective, the use of radiotracers for brain scanning began in the early 1950s for the localization and identification of tumor damage by Sweet and Brownell [1–3].

Several compounds incorporating radionuclides were synthesized in order to obtain stable agents with a low toxicity profile. Among these, the most satisfactory have been the positron emitters arsenic-74 as arsenate, copper-64 as versenate, and mercury-203 as neohydrin [4, 5]. These scanning agents were not required to have any specific interaction; they were able to permeate across the disrupted blood–brain barrier (BBB), thus allowing a rudimentary detection of the membrane integrity and the lesion extensions with camera-type systems. Despite the raising interest for the methodology, these long half-life radionuclides displayed suboptimal characteristics for a clinical use. A few years later, driven by favorable nuclear properties, a radionuclide generator, and known chemistry, the researcher's attention shifted to the development of a suitable agent containing gallium-68 [6]. As a consequence of the available “ready to use” ^{68}Ga -EDTA (ethylenediaminetetraacetic acid) milked from the generator, and the introduction of new positron scintillator cameras [7, 8], hundreds of patients were investigated during the 1960s [9, 10]. Unfortunately, the enthusiastic consensus for gallium-68 imaging faded away during the 1970s, mainly because of the restricted use of first-generation $^{68}\text{Ge}/^{68}\text{Ga}$ generators and the parallel development of new emerging radionuclide tracers, such as single-photon emitters technetium-99m/iodine-123 and the positron emitting carbon-11/fluorine-18.

Since the dramatic change that occurred at Brookhaven National Laboratory in the late 1950s for the availability of $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generators, the radiometal technetium-99m opened a new era in Nuclear Medicine as well as in early neuroimaging [11]. The ideal nuclear properties, the easy availability from a generator, and the flexible chemistry facilitated the introduction of this emerging radionuclide that is, at present, the most employed for Nuclear Medicine procedures worldwide. The first agents $^{99\text{m}}\text{Tc}$ -pertechnetate and $^{99\text{m}}\text{Tc}$ -DTPA (diethylenetriaminopentaacetic acid), as well as other gamma emitters radiotracers thallium-201 and gallium-67 citrate, were evaluated for pathologies with altered BBB due to tumors or traumas [12–14]. However, all these

radiotracers had the relevant constraint that they could be employed in neurological disease just for the evaluation of an altered BBB.

In the late 1950s, a few years later from the initial brain tumor scanning, Munck and Lassen pioneered the assessment of cerebral blood flow (CBF) by using inert radioactive gases, thus providing the basis for regional CBF functional imaging. Evaluation in human brains was performed by administering trace amounts of krypton-85 by inhalation or intravenous injection in saline solution [15, 16]. In the original methodology, blood samples were collected from bilateral jugular veins and counted, reflecting the global blood flow and oxygen use. The further advance was the measurement of the regional cerebral perfusion achieved by placing detectors on the patient's scalp, to determine radiotracer accumulation and clearance on a regional basis, thus opening the way for a more modern concept of CBF scanning using a noninvasive approach [17, 18].

In the 1960s, major improvements occurred using molecular [^{15}O]oxygen for regional oxygen extraction and perfusion assessments, also taking advantage of the increased performances of imaging systems due to the introduction of first-generation Anger cameras [19]. Ter-Pogossian and Brownell evaluated the radiotracer kinetics after a single breath by means of a pair of detectors. However, the first results were not satisfactory because of the difficult interpretation of the collected data [20, 21]. Some years later, radioactivity administration was modified by using continuous [^{15}O]oxygen inhalation, producing a “steady-state” brain distribution dependent on perfusion and oxygen extraction, as well as the physical decay of the radioisotope [22]. Nevertheless, this technique suffered for some important disadvantages such as a constant delivery of the radioactive gas and a long-time scan during which, it is assumed, no change in physiological status occurs. A more practical and suitable method for imaging was then introduced using an intravenous injection of [^{15}O]oxygen water instead of a labeled gas [23], becoming a standard procedure for rCBF assessment with PET. These methods have been used in clinical

settings since the 1980s in several clinical conditions including strokes, brain tumors, and Parkinson's disease [24–26]. Despite the fact that the early evaluations were much more qualitative than quantitative, these methodologies represent a milestone for neuroimaging because of the direct impact on patient healthcare.

An alternative to PET radiopharmaceuticals for the rCBF measurement was developed during the 1980s by the parallel and successfully investigational studies with iodine-123 and technetium-99m. These efforts resulted in the development of perfusion agents able to overpass the normal BBB, such as [^{123}I]iodoantipyrine, [^{123}I]IMP (1-3-[^{123}I]iodo- α -methyl-tyrosine), [$^{99\text{m}}\text{Tc}$]HMPAO ([$^{99\text{m}}\text{Tc}$]exametazime), and [$^{99\text{m}}\text{Tc}$]ECD ([$^{99\text{m}}\text{Tc}$]bicisate). Most of them have reached an established clinical use for the evaluation of various neurological diseases such as dementia, Alzheimer's disease, epilepsy, stroke, and Parkinson's disease [27–30].

A fundamental advance for Nuclear Medicine applications was the application of fluoro-18-deoxyglucose ([^{18}F]FDG) to image cerebral glucose metabolism in 1976, destined to become the most important PET tracer to this day with indications for tumors and for identification of foci of epileptic seizures [31, 32]. [^{18}F]FDG, a glucose analog, is able to delineate the glucose metabolism, which is very active in the normal brain and often hyperreactive in tumors.

The promising results using a radiotracer able to interact with substrates, in addition to the early quantitative imaging applications, confirmed the full potential of this emerging technique in describing molecular processes on human brain in health and disease. A general enthusiasm and great expectation for the future were soon satisfied by a rapid increase in the development of new radioactive molecules, typically including the positron emitters carbon-11 and fluorine-18 and single-photon emitters iodine-123 and technetium-99m. The new generation of radiotracers were designed for displaying affinity for receptors, enzymes, or other biological structures thus defined as radioligands, for interacting with specific metabolic pathways or for reproducing the chemical structures of drugs incorporating a radionuclide with identical characteristics or with

minimal modifications or at least retaining the key biochemistry. Among the expanded range of imaging molecules, the first widespread applications included the regional amino acid metabolism using native amino acids or derivatives labeled with carbon-11, like [^{11}C]methionine, leucine, and unnatural amino acids [33–35]. A few years later, fluorine-18 dihydroxyphenylalanine ([^{18}F]FDOPA), a dopamine synthesis pathway analog, and N-[^{11}C]methylspiperone, a radioligand for dopamine/serotonin receptors, were used to assess for the first time the neurotransmission function in humans [36, 37]. The mid- to late 1980s were characterized by a continuous introduction of new radioligands with major advances for the quantification of dopamine receptor subtypes ([^{11}C]raclopride, [^{11}C]Schering-23390), dopamine transporters ([^{11}C]nomifensine), central benzodiazepine receptors ([^{11}C]flumazenil), peripheral benzodiazepine receptors ([^{11}C]PK11195), opioid receptor agonists and antagonists ([^{11}C]carfentanil and [^{11}C]diprenorphine) [38–44], as well as for the enzyme monoamine oxidase type B ([^{11}C]deprenyl) [45]. A new field of research appeared to be very auspicious for more specific and useful clinical applications with PET chemistry imposing a dominate role in neuroimaging.

Radiotracer expansion certainly benefitted from an increased understanding of both physiological and pathological molecular processes, but the great effort of chemists, pharmacists, biologists, physicists, and physicians was key to the progress. The influx of scientists from various fields allowed the discovery and validation of new radiopharmaceuticals to achieve a vast array of targeted radiotracers. This great evolution was also facilitated by the increased availability of supporting technologies and infrastructures such as generators, cyclotrons, SPECT and PET scanners, and chemistry laboratories. In addition, the notable improvements in radiochemistry, mainly in labeling strategies for rapidly labeling molecules with high specific activity starting from the most popular short-life PET radionuclides, have boosted the design of new radiotracers claiming the important role this discipline is playing in this field.

During the 1990s, a progressive investigation focused on the improvement of the existing imaging, as well as in modulating and delineating neurotransmission systems other than dopaminergic and serotonergic. [^{11}C]WAY-100635, [^{11}C]MDL-100907, and [^{11}C]McNeil-5652 were introduced, respectively, for a more selective evaluation of serotonin receptor subtypes 1a/2a distribution and for the SERT/5-HTT transporters [46–48]. Novel [^{11}C]NNC112, [^{11}C]FBL457, and [^{11}C]- β -CIT displayed high affinities, respectively, for dopamine receptor subtypes 1 and 2/3 and for dopamine transporters [49–51]. Beyond the dopaminergic/serotonergic systems, new imaging agents were aimed at exploring acetylcholinesterase enzyme ([^{11}C]MP4A and [^{11}C]PMP) and nicotinic receptor reserves (2-[^{18}F]F-A85380), as well as vesicular monoamine transporter type 2 ([^{11}C]DTBZ) and ATP-binding cassette (ABC) transporters like P-glycoprotein ([^{11}C]verapamil) [52–56].

For many years, the development of target-specific agents has been strongly dominated by PET chemistry, given the relatively easy replacement of a carbon, an oxygen or nitrogen with a radioisotope conserving the original chemical structures. However, the major worldwide diffusion of SPECT scanners promoted the investigation for the development of agents containing a suitable single-photon emitter radionuclide. A number of radioiodinated tracers which bind CNS receptors were synthesized and introduced successfully as alternatives to PET tracers in clinical settings. Iodinated derivatives displayed excellent properties for the quantitative evaluation of the muscarinic ([^{123}I]IQNB, [^{123}I]iododexetimide), dopamine ([^{123}I]IBZM and [^{123}I]epidepride), and benzodiazepine ([^{123}I]iomazenil and [^{123}I]NNC-13-8241) receptor reserves and for dopamine/serotonin transporter function ([^{123}I]- β -CIT) [57–63]. Because of the intrinsic difficulties to incorporate a radiometal such as technetium-99m into small molecules, able to pass the normal BBB and conserving the affinity for a specific target, investigational studies were not as consistent as for the main PET tracers in neurology. The synthesis and evaluation of the first [$^{99\text{m}}\text{Tc}$]-labeled

tropane analogs that display selective dopamine transporter binding were reported in 1996 and 1997 (TRODAT-1 and technepine), and [$^{99\text{m}}\text{Tc}$]TRODAT-1 has succeeded in entering evaluation for clinical approval a few years later [64–66].

From 2000 to the present, the range of radiotracers for studying biochemical and pathophysiological molecular mechanisms has been expanding continuously. New targets of interest for neurological applications have been explored, among which are norepinephrine transporters ([^{11}C]methyl reboxetine), neurokinin-1 ([^{18}F]SPA-RQ) and cannabinoid-1 ([^{18}F]MK-9470) receptors and Alzheimer-related proteins (beta-amyloid with [^{11}C]PiB (Pittsburgh compound B) and related fluorine analogs, and more recently tau protein and alpha-synuclein with [^{18}F]THK5105) [67–71]. The concomitant advances in PET technology and the scanner proliferation in industrialized countries corroborated to establish the role of imaging in both research and clinical settings to improve our understanding in diagnoses, monitoring disease progression, and the response to treatment.

Unfortunately, relatively few radiotracers have been translated into the clinical setting, due to high requirements and complex procedures for getting the approval from authorities and, not least, because of the high costs of the required infrastructures. In addition, the utility of carbon-11, incorporated in most of the developed compounds, is limited by its short radioactive half-life, and for this reason, its on-site production and use are essential. On the contrary, fluorine-18 radiotracers may be produced and distributed to local hospitals, making use of this technology more widely accessible. A notable and successful example on the evolution for a specific clinical need, although uncommon, is represented by the radiopharmaceuticals employed in amyloid PET imaging. The first published experimental data using a carbon-11 compound ([^{11}C]PiB) with affinity for amyloid plaques has been followed by the development and commercialization of fluorine-18 analogs ([^{18}F]florbetapir, [^{18}F]florbetaben, and [^{18}F]flutemetamol) in less than 10 years [72].

It is expected that in the future, brain imaging will continue to benefit from new radiotracers and radiopharmaceuticals, although research activities appear to be less optimistic with respect to the initial enthusiastic consensus [73]. However, the lack of appropriate radiotracers for unknown molecular brain mechanisms represents one of the major challenges for radiochemists in supporting Nuclear Medicine imaging to understand physiological processes and diseases and for application to drug development.

4.3 Radiotracer and Radiopharmaceutical Design for the Development of Brain Imaging Agents

From the 1920s, when George Charles de Hevesy coined the term “radiotracer” paving the way for the application in biomedical sciences, many imaging agents have been developed and applied in Nuclear Medicine [74]. Over the years, brain imaging studies have evolved to include single-photon-emitting (SPECT) and positron-emitting (PET) radiopharmaceuticals particularly used in the evaluation of brain tumors, central motor disorders, and cognitive disorders.

Based on their mechanism of action, SPECT and PET brain radiotracers are generally classified in two groups according to their capability to perfuse cerebral tissues or to interact with specific substrates. The first group comprises the substrate nonspecific agents able to cross BBB and trace dynamic processes for high-capacity systems. They represent an important class of radiotracers, which biodistribution correlates with cerebral blood flow or brain tissue permeability; thus, they are also defined as perfusion agents (e.g., [^{99m}Tc] HMPAO and PET [^{15}O]water).

The second group is referred to those radiotracers with specificity for biochemical targets susceptible to changes as function of disease states. These agents are dependent on the densities of the interested molecular targets such as

receptors, enzymes, and transporters. Since they are usually present at very low concentrations and are susceptible to saturation, these targets are defined as low-density-easily saturated sites. Lately, a major interest has been devoted to this class of radiotracers, due to their capability of detecting targets at the molecular level.

Despite the relevant results achieved in research during the last decades, the range of radiotracers and radiopharmaceuticals used for brain imaging in clinical settings worldwide appears limited (Table 4.2). In 2013, Molecular Imaging and Contrast Agent Database (MICAD) reported 11 SPECT and almost 100 PET tracers that have been tested at least once in humans, with potential as brain imaging agents [75]. However, a very few of them have reached the clinical state. This is consistent with the general reduced trend of approval of new pharmaceuticals (including radiopharmaceuticals) by the National Regulatory Authorities responsible for human medicines that shows a constant decline in approvals after 2000. Regulatory aspects and clinical development have to be considered the main reasons for such a few introductions, together with the economic aspects. An estimate of time and costs for the clinical development of diagnostic agents has been reported by Nunn and more recently by Zimmermann, who calculated that a radiopharmaceutical has a cost of €20–60 million from the time the lead molecule is identified and takes 7–9 years to develop (Fig. 4.1) [76, 77]. A conventional therapeutic drug has a higher cost, approximately €82–300 million to develop over 10–15 years, but the related market is by far larger than that of imaging agents, thus generating a much higher return on investment [78]. It is therefore evident that the commercialization of diagnostic imaging agents will not generate the same income in a comparable amount of time.

In this scenario, academic investigators play a major role in the design and synthesis of diagnostic imaging agents whose development has to be in line with the emerging needs of the public

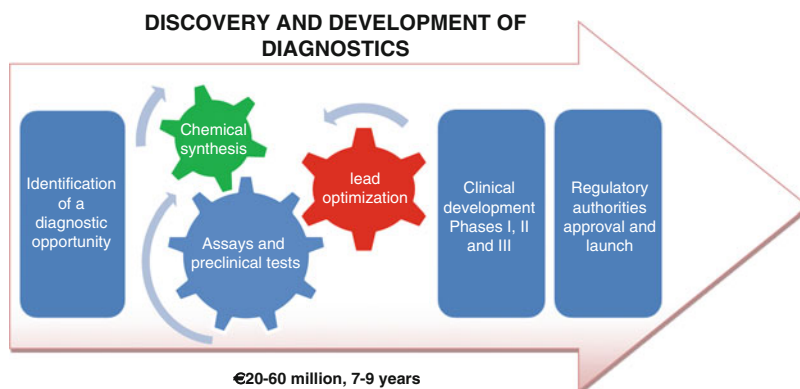
Table 4.2 Radiopharmaceuticals approved or registered by federal authorities

Common name	Common names, acronyms, and nomenclature	Process/target
<i>Flow and perfusion</i>		
[^{99m} Tc]ECD	[^{99m} Tc]-Bicisate	Metabolic trapping
[^{99m} Tc]HMPAO	[^{99m} Tc]-Exametazime	Metabolic trapping
[¹⁵ O]O ₂		Oxygen diffusion
[¹⁵ O]H ₂ O		Water diffusion
[¹⁵ O]CO		Red blood cell labeling
[^{81m} Kr]Krypton		Diffusion
[¹³³ Xe]Xenon		Diffusion
<i>Tumor metabolism</i>		
[¹⁸ F]FDG	2-Deoxy-2-fluoro-D-glucose	Glucose metabolism
[¹²³ I]IMT	3-Iodo-L-α-methyl tyrosine	Amino acid transport
[¹¹ C]Methionine	(S)-2-Amino-4-(methylthio)butanoic acid	Amino acid transport
[¹⁸ F]FET	Fluoroethyltyrosine	Amino acid transport
[¹¹ C]Choline	2-Hydroxy-N,N,N-trimethylethanammonium	Cell membrane metabolism
[¹⁸ F]Methylcholine	2-Fluorooxyethyl(trimethyl)azanium	Cell membrane metabolism
[¹¹ C]Acetate	[1- ¹¹ C]Acetate	Lipid metabolism
[¹⁸ F]FLT	3'-Deoxy-3'-fluorothymidine	DNA synthesis
[¹⁸ F]FMISO	Fluoranyl-3-(2-nitroimidazol-1-yl)propan-2-ol	Hypoxia
[^{99m} Tc]MIBI	[^{99m} Tc]-Sestamibi	P-glycoprotein
[²⁰¹ Tl]Thallium chloride		Potassium analog
<i>Dopamine system</i>		
[¹⁸ F]FDOPA	(2S)-2-amino-3-(2-fluoranyl-4,5-dihydroxyphenyl)propanoic acid	Dopamine synthesis
[¹²³ I]FP-CIT (DaTscan)	Ioflupane(123I) 2-Carbomethoxy-8-(3-fluoropropyl)-3-(4-iodophenyl)tropane	Dopamine transporter
[¹²³ I]E-IA-F-CIT	Altropane(123I) N-Iodoallyl-2-carbomethoxy-3-(4-fluorophenyl)tropane	Dopamine transporter
[^{99m} Tc] TRODAT-1	2-[2-[(3S,4R)-3-(4-chlorophenyl)-8-methyl-8-azabicyclo[3.2.1]octan-4-yl)methyl-(2-sulfidoethyl)amino]acetyl]azanidylethanethiolate;oxo- technetium-99	Dopamine transporter
[¹²³ I]Beta-CIT(DOPAScan)	Iometopane(123I) 2Beta-carbomethoxy-3beta-(4-iodophenyl)tropane	Dopamine transporter
[¹²³ I]Epidepride	N-[[[(2S)-1-ethylpyrrolidin-2-yl]methyl]-5-iodo-2,3-dimethoxybenzamide	Dopamine D2/D3 receptor
[¹²³ I]ADAM	2-[2-[(Dimethylamino)methyl]phenyl]sulfanyl-5-iodaniline	Serotonin transporter
[¹¹ C]Raclopride	(S)-3,5-Dichloro-N-((1-ethylpyrrolidin-2-yl)methyl)-2-hydroxy-6-methoxy-(S)-3,5-dichloro-N-((1-ethylpyrrolidin-2-yl)methyl)-2-hydroxy-6-methoxybenzamide	Dopamine D2 receptor
[¹²³ I]IBZM	Iolopride(123I) N-[[[(2S)-1-Ethylpyrrolidin-2-yl]methyl]-2-hydroxy-3-iodanyl-6-methoxybenzamide	Dopamine D2 receptor

(continued)

Table 4.2 (continued)

Common name	Common names, acronyms, and nomenclature	Process/target
N-[¹¹ C]Methylpiperone	8-[4-(4-Fluorophenyl)-4-oxobutyl]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5] decan-4-one	Dopamine D2/5-HT ₂ receptor
<i>Receptor- and enzyme-binding ligands (other than for Dopamine system)</i>		
[¹¹ C]PK11195	1-(2-Chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinolinecarboxamide	Translocator protein (TSPO)
[¹¹ C]Flumazenil	4H-Imidazo(1,5-a)(1,4)benzodiazepine-3-carboxylic acid, 8-fluoro-5,6-dihydro-5-methyl-6-oxo-, ethyl ester	GABA receptor
[¹²³ I]Iomazenil	Ethyl 7-iodo-5-methyl-6-oxo-4H-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate	GABA receptor
2-[¹⁸ F]F-A85380	2-Fluoro-3-[2(S)-2-azetidylmethoxy]pyridine	nACh receptor
[¹¹ C]MP4A	N-[¹¹ C]Methylpiperidin-4-yl acetate	AChE activity
<i>Plaque-binding ligands</i>		
[¹¹ C]PiB	2-(4'-[¹¹ C]Methylaminophenyl)-6-hydroxybenzothiazole	Plaque acceptor
[¹⁸ F]Florbetapir (Amyvid)	4-[(E)-2-[6-(2-{2-[2-(18F)Fluoroethoxy]ethoxy}ethoxy)-3-pyridinyl]vinyl]-N-methylaniline	Plaque acceptor
[¹⁸ F]Flutemetamol (Vizamyl)	2-[3-(18F)Fluoro-4-(methylamino)phenyl]-1,3-benzothiazol-6-ol	Plaque acceptor
[¹⁸ F]Florbetaben (Neuraceq)	4-[(E)-2-[4-(2-{2-[2-(18F)Fluoroethoxy]ethoxy}ethoxy)phenyl]vinyl]-N-methylaniline	Plaque acceptor
<i>Cisternography</i>		
[¹¹¹ In]Indium diethylenetriamine pentaacetic acid injection	Sodium;2-[bis[2-[bis(carboxylatomethyl)amino]ethyl]amino]acetate;indium-111	CSF transport

**Fig. 4.1** Discovery and development process for a diagnostic (Adapted from FDA's Critical Path Initiative, cost and timeline numbers taken from Nunn and Zimmermann)

health system, but must be also economically sustainable for the diagnostic companies.

It is remarkable that within the few approved SPECT and PET agents in the last decade, most of them are brain imaging radiopharmaceuticals, with a tremendous increase of interest in the devel-

opment of PET tracers for the diagnosis of neurodegenerative disorders. This shows that the current efforts, both from academia and industry, point toward a more accurate and early diagnoses of CNS pathologies in order to help clinical decisions that improve the effectiveness of medical care.

4.3.1 Radiopharmaceuticals for High-Capacity Systems or for Low-Density-Easily Saturated Sites

The design and the development of a radiopharmaceutical are strictly connected with its application: delineating a pathophysiological process or targeting a specific molecular structure.

The majority of the technetium-99m radiopharmaceuticals currently in use have been designed and developed for high-capacity systems. They are formulated from commercially available “instant kits” and pertechnetate-99m obtained from $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generators. Each kit typically contains the ligand, the radionuclide has to be complexed, and an adequate quantity of reducing agent buffers to adjust the pH and to suit the labeling conditions, stabilizing agents and excipients. The radiotracer is thus obtained from the reaction between the ligands and the radionuclide in an appropriate oxidation state, resulting in a new stable coordinated system. The integral chemical structure for each complex, composed by the radionuclide core and ligands, is responsible for pharmacokinetic and pharmacodynamic properties. $[^{99\text{m}}\text{Tc}]\text{HMPAO}$ and $[^{99\text{m}}\text{Tc}]\text{ECD}$ are examples of kit-formulated radiopharmaceuticals for brain imaging routinely prepared in Nuclear Medicine worldwide [29, 30]. However, the amount of ligands contained in an “instant kit” is by far greater than that of technetium-99m, and it would saturate many low-capacity targets. This is the reason why these radiopharmaceuticals are primarily used for the imaging of high-capacity systems, such as regional cerebral blood flow, where saturation cannot be achieved. $[^{99\text{m}}\text{Tc}]$ -kit-based radiopharmaceuticals are covering over 80 % of all Nuclear Medicine diagnostic procedures worldwide, with a noteworthy contribution to neuroimaging studies, although receptors and other molecular structures are subjects of major interest in brain imaging research at present.

Radiolabeled agents with high specificity for low-density-easily saturated sites are usually modeled on partial agonists and antagonists in the CNS therapeutic drug repertoire and lead compounds in the field of pharmaceutical

research. Once the chemical structure of a potential new radiotracer has been identified for a specific biological target, the next step is to synthesize the desired compound by coupling an emitting nuclide suitable for imaging.

Many radiolabeled compounds with selectivity for receptors, enzymes, and transporters both for SPECT and PET brain imaging have been developed over more than 40 years of research. Among these, radiotracers based on positron emitters provide much useful information on neurophysiology and neuropharmacology of cholinergic, serotonergic, dopaminergic, GABA/benzodiazepine, opioid, and other neurotransmission systems [73]. Furthermore, PET methodology offers several advantages over SPECT, such as a higher sensitivity, better temporal and spatial resolution, shorter imaging protocols, and a lower patient dosimetry [79]. On the other hand, PET needs cyclotron and radiopharmacy facilities, which are expensive and require highly skilled personnel to operate.

SPECT radiotracers for low-density-easily saturated sites have a long history of radiochemistry research due to the relatively easy labeling with iodine-123 and the large availability of technetium-99m. A number of single-photon-emitting radiotracers for brain imaging have reached the clinical state and are commercially available in many countries. SPECT methodology is by far largely widespread compared with PET; therefore, it still plays a fundamental role allowing access to neuroimaging examinations in many conventional Nuclear Medicine centers worldwide. Recent technological improvements have reduced the gap between SPECT and PET [80], thus promoting the research and development of new single-photon-emitting radiotracers to satisfy the clinical need in Nuclear Medicine which are not supported by a PET cyclotron facility.

4.3.2 Challenges in Brain Imaging Radiotracer Development

The development of new diagnostic agents for brain imaging follows two main initial steps:

first the identification of the biological process indicative of the pathology to be investigated and second the identification of the molecular target best representing the process. This target will virtually represent the chemist's workbench for the design of the probe chemical structure.

During the pregenomic era, radiotracer design was based on *in vitro* assays, *in vivo* biodistribution studies, and autopsy data. This approach has been successful for a number of SPECT and PET agents with specificity for targets at molecular level, including most of the commonly applied radiopharmaceuticals (metabolic and receptor-binding tracers), but it does not represent the ideal approach for the design of early diagnostic agents [81].

However, considerable advances in molecular cell biology and the sequencing of the human genome opened the way for the postgenomic era with the advent of the new millennium. Ever since, new potential drug targets, as well as biomarkers, have become available from the genomics, proteomics, and single nucleotide polymorphisms (SNPs) findings [82]. Molecular imaging, as other fields in medicine, has gained benefit from the identification of new targets. This, together with the concomitant extended repertoire of tools and technologies, promoted the design of novel imaging probes for the application in Nuclear Medicine moving toward "precision medicine." In the future, the personalized imaging probes are expected to guide the treatment for patients (especially targeted treatments) and to significantly improve healthcare delivery and reduce its costs.

In this scenario, radiochemistry has maintained its crucial role, and the design and synthesis of novel radiotracers, which may become radiopharmaceuticals in clinical settings, are still considered one of the major challenges for radiopharmaceutical chemists.

The development of brain imaging radiotracers is not easy to carry out, especially if compared to other diagnostic agents, due to the extraordinary complexity of cerebral anatomy and biochemical functions [83, 84]. Radiotracer's pipeline has a typical bottleneck shape, where

only a few of the numerous candidates synthesized will show suitable *in vivo* properties and eventually become radiotracers and then radiopharmaceuticals. When designing SPECT and PET radiotracers, the following key factors must be taken into account:

- Choice of the target indicative or representative of the pathology under investigation with consideration of an unmet need in the diagnosis of this pathology
- Identification of the lead compound/structure with specificity for the target
- Selection of the radionuclide
- Labeling strategy to synthesize the desired chemical structure

In addition, in order to allow the radioactivity to distribute within the brain and to quantify it with imaging techniques, radiotracers should ideally exhibit:

- Rapid and quantitative permeation through the blood–brain barrier (BBB)
- High affinity and selectivity for the target
- *In vivo* stability and absence of radioactive metabolites
- Favorable pharmacokinetic and pharmacodynamic properties in relation to radionuclide half-life

Other factors should also be considered in respect to the pharmaceutical requirements for human use, imposed by Drug Regulatory Authorities. Current radiopharmaceutical development shares much with standard drug discovery and development practices, and although diagnostic radiopharmaceuticals are generally administered only a few times in a patient's lifetime and the adverse reactions are extremely rare, the safety profile of a new radiotracer has to be demonstrated and validated. This assessment includes the evaluation of pharmacological and toxicological activity of the diagnostic preparation (including any component other than the labeled tracer) and the estimation of the absorbed radiation dose to prove a favorable risk-to-benefit ratio.

Last but not least, researchers should also keep in mind that, to be successful, radiopharmaceutical development should satisfy a specific clinical need with direct impact on patient disease treatment [85]. Although effectiveness of a radiotracer is required by regulatory authorities in order to get approval, a clear diagnosis of a pathologic status is most of the times difficult to achieve with brain imaging agents. An example is depicted by the recently introduced PET radiopharmaceuticals for quantitative imaging of beta-amyloid plaques [86]. Due to the occurrence of beta-amyloid deposition in normal elderly people and in clinical syndromes other than Alzheimer's disease (AD), amyloid quantification is an ambiguous indicator, even though plaques are one of the defining pathologic features of AD. Clinical trial studies have been designed to validate PET amyloid technology and to understand disease mechanisms, rather than to find new applications in clinical practice for an effective and early AD diagnoses. Therefore, the use of amyloid PET to clinically improve AD outcomes and to help medical decision-making has been long debated [87].

Recent efforts of researchers are aimed at identifying single targets that could be used for revealing and monitoring the progression of diseases. The discovery of new biomarkers for the accurate identification of a pathologic status will certainly assist radiochemists in their efforts to design and develop novel radiolabeled agents.

Since an ideal model of radiotracer development does not exist, we will now illustrate the main concepts for radiotracer design and development and their use for brain imaging, based on the research experience and some examples of successful applications in clinic settings.

4.3.3 True Radiotracers, Isotopic Radiotracers, and Analog Radiotracers

Labeling a desired biomolecule is not always an easy task. Since CNS molecules are always small in size (<500 Da) to cross the normal BBB, labeling may have a detrimental effect on the in vivo

properties of the biomolecule and, eventually, on the final quality of the diagnostic image. Therefore, in the design of a new brain imaging radiotracer, particular attention has to be paid to the labeling strategy to obtain the desired molecular structure.

The process of coupling the radionuclide to the biomolecule can be achieved via the formation of covalent bonds for nonmetallic radioisotopes (such as carbon, nitrogen, oxygen, and the halogens) and dative bonds for metallic radionuclides (as technetium-99m or Indium-111).

The replacement of endogenous elements, such as carbon-12, oxygen-16, or nitrogen-15 with a radioisotope, is an appealing solution to avoid molecular perturbation due to the insertion of a radionuclide. Labeled compounds synthesized via isotopic substitution of organic molecules are defined as "true tracers," and their biochemical properties, including pharmacokinetics and pharmacodynamic properties, do not differ from those of the original biomolecule. [¹¹C]methionine, [¹¹C]acetate, and L-β-[¹¹C]DOPA are examples of true tracers (Fig. 4.2). The most important peculiarity of true radiotracers is their ability to study the components of a physiologic or pathologic process without disturbing the homeostatic system.

Isotopic substitution is also a convenient labeling approach for exogenous molecules with known pharmacologic activity, such as CNS drugs. In addition to the replacement of naturally occurring elements, other isotopes can be considered, for example, halogens. Fluorine-19 is not typically contained in endogenous molecules, but it is a common constituent of many pharmaceuticals, due to its contribution to improve drug bioavailability. Replacement with fluorine-18 can generate an "isotope tracer" identical to the chosen lead compound, which shares the same in vivo behavior without showing any pharmacological effect. The chemical structure of flumazenil, for example, allows the preparation of two isotope tracers: the carbon substituted [¹¹C]flumazenil ([¹¹C]FMZ) and the fluorine substituted [¹⁸F]flumazenil ([¹⁸F]FFMZ) (Fig. 4.2). They are both isotopic tracers of flumazenil with similar kinetic and binding behaviors, but with different

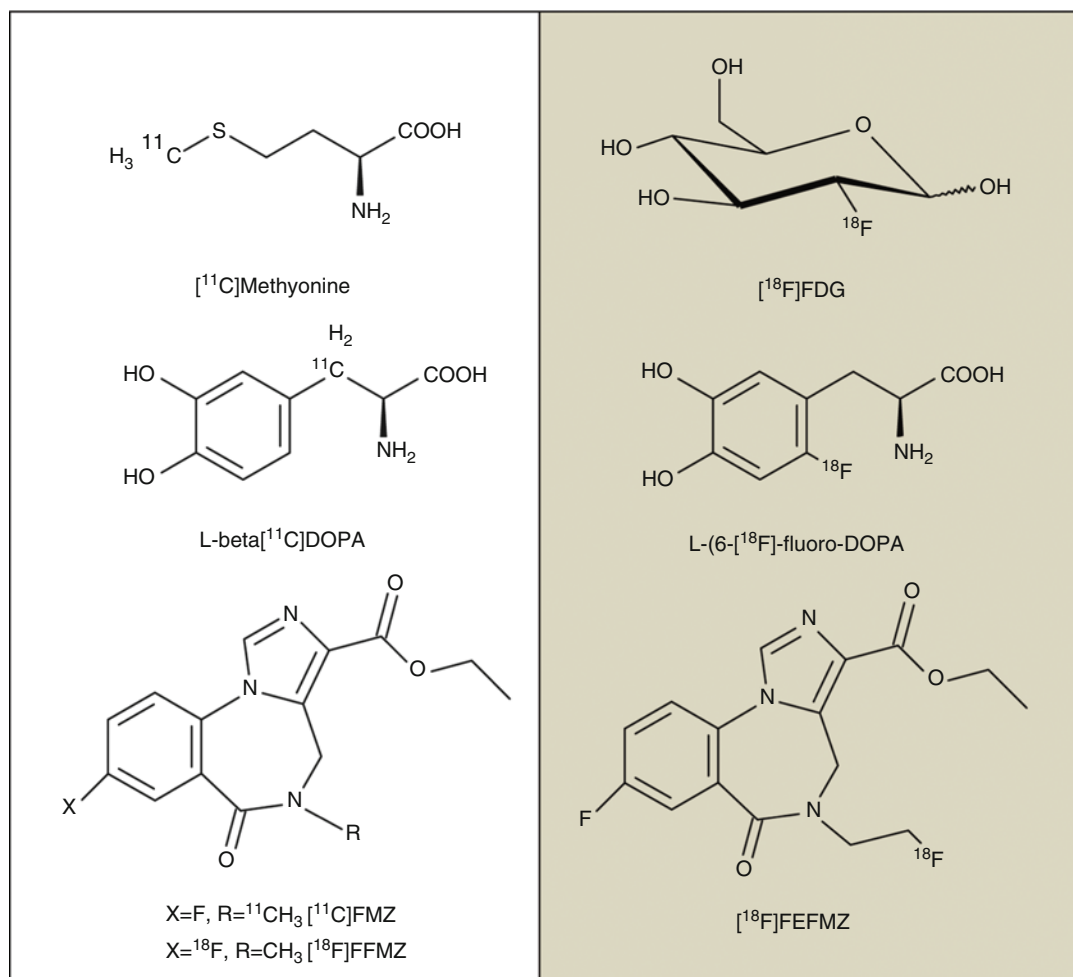


Fig. 4.2 Chemical structures for selected true tracers (and isotopic tracers) on the *left* and analog tracers on the *right*

radionuclide half-lives and perhaps different radioactive metabolites [88].

If the chosen radionuclide for radiolabeling does not have a stable isotope in its structure or it is not chemically convenient to replace, isotopic substitution is not an adequate strategy. This situation is extremely common, and to overcome this problem, labeling may be achieved by a chemical modification of the tracer structure, generating a derivative known as an “analog tracer.” In order to assure a minimal molecular perturbation, substitution with a radiochemical scaffold of similar steric or electronic characteristics to the original molecule should be preferred. However, due to the labeling, the final radioactive molecule will be

different from the native compound, and its binding affinity and selectivity for the target, as well as pharmacokinetic and pharmacodynamic properties, need to be tested for confirming its potential use as an imaging agent. For example, while L-β[¹¹C]DOPA is a true tracer for L-DOPA, [¹⁸F]FDOPA L-(6-[¹⁸F]-fluoro-DOPA) is an analog [¹⁸F]-tracer, which differs for an additional fluorine in position 6 of the aromatic ring (Fig. 4.2). They are both used as biochemical probes to assess the rate of endogenous dopamine synthesis, although they have a different metabolism [89].

The biological behavior of an analog tracer is difficult to predict. However, considering the general small dimension of the interested

molecules, a modification in the chemical structure is often followed by an alteration of binding affinity and/or pharmacokinetic properties. A relevant number of studies demonstrating the variable influence of chemical modifications, due to labeling with different strategies, are reported in literature for many PET and SPECT radiotracers. Many analog tracers are produced by incorporating [^{18}F]alkyl scaffold of different lengths on N-, O-, or S-functions of the parent molecules. While N-[^{18}F]fluoroethyl flumazenil ([^{18}F]FEFMZ) maintained the specific binding to the benzodiazepine receptor, an N-[^{18}F]fluoropropyl derivative lost completely this affinity [90]. On the contrary, among the [^{18}F]-labeled spiperone analogs N-(2-[^{18}F]-fluoropropyl)-spiperone, the longer N-alkylated derivative showed improved binding and kinetic properties in comparison with N-(2-[^{18}F]-fluoroethyl)-spiperone [91].

In some special cases, analog tracers are desirable because they exhibit superior characteristics as imaging agents in comparison with true tracers, due to their *in vivo* behavior. A prominent example is depicted by [^{18}F]FDG, the most important analog tracer used for the assessment of glucose metabolism (Fig. 4.2). Once [^{18}F]FDG and glucose entered in the cell, they can either be transported back to the plasma or, as first metabolic step, they can equally be phosphorylated by hexokinase. In contrast, glucose continues along the glycolytic pathway for energy production while [^{18}F]FDG cannot enter glycolysis and becomes effectively trapped as FDG-6-phosphate, thus reaching a steady state. Therefore, [^{18}F]FDG is susceptible to metabolic trapping thus facilitating a PET study, while a glucose true tracer with carbon-11 or oxygen-15 produces many different radioactive metabolites, resulting in the need of image correction with mathematical models.

4.3.4 Common Radionuclides for Brain Imaging and Related Labeling Strategies

4.3.4.1 Carbon-11

Carbon-11, a positron emitter, is considered to be the preferable radionuclide for true tracer design,

given the abundance of carbons in endogenous and exogenous molecules and the virtual versatility of organic chemistry labeling reactions. On the other hand, its fast decay (20.4 min) represents a major constraint in the clinical practice and imposes several requirements in the preparation and use of [^{11}C]-labeled compounds such as the presence of an in-house cyclotron and the development of a rapid procedure of radiolabeling, purification, and quality controls. As a rule of thumb, in order to be sustainable for clinical use, the production and release of [^{11}C]-radiotracers should be carried out within three half-lives of the radionuclide. This will afford manipulating enough radioactivity to allow the injection, distribution of the radionuclide, and the patient scanning according to the imaging protocol.

[^{11}C]-PET tracer production is currently based on the conversion of the irradiated in-target product [^{11}C]carbon dioxide or methane ([^{11}C]CO₂ or [^{11}C]CH₄) to a secondary reactive precursor (or synthon) that ensures labeling of suitable substrates in few minutes and with high yields. [^{11}C]methyl iodide and [^{11}C]methyl triflate are the most prominent examples of synthons for nucleophilic [^{11}C]methylation of primary and secondary alcohols, amines, and thiols, commonly applied for the production of many [^{11}C]radiotracers and radiopharmaceuticals, such as [^{11}C]methionine, [^{11}C]choline, [^{11}C]PIB, [^{11}C]raclopride, N-[^{11}C]methylspiperone, [^{11}C]carfentanil, and [^{11}C]McN-5652 [92]. When simple methylation is no longer a viable approach, the radionuclide has to be incorporated into the molecule with a different labeling strategy. [^{11}C]C-C bond formation is often required, especially for labeling molecules with functional groups such as carboxylic acids, ketones, amides, and imides. A common procedure is based on the direct reaction of target-prepared [^{11}C]CO₂ with a Grignard reagent to produce an intermediate, which can be hydrolyzed to give [carbonyl- ^{11}C]carboxylic acids or converted to [carbonyl- ^{11}C]amides [93]. In recent approaches, [^{11}C]carbonylation is also achieved through transition-metal-mediated reactions using [^{11}C]

methyl iodide, [^{11}C]cyanide, or [^{11}C]carbon monoxide, as secondary precursors. Several palladium-mediated cross-coupling reactions have been shown to be effective and very innovative approaches for preparing many [^{11}C] tracers. However, despite the availability of a large variety of synthetic strategies for [^{11}C]-radiotracers and radiopharmaceuticals, the majority of their production scale for clinical routine is limited to a few of the reactions described above [92, 93].

4.3.4.2 Fluorine-18

Another positron emitter widely used for the preparation of brain imaging agents is fluorine-18, the most important radionuclide for PET imaging. As mentioned above, fluorine is not typically present in endogenous molecules, in contrast to carbon and the most common heteroatoms. Its exceptional employment for in vivo imaging is primarily due to the minimal perturbation caused by its incorporation (similar to a hydroxyl substituent) into the final molecule, combined with advantageous physical half-life of 109.7 min, which permits multistep syntheses as well as a major flexibility in postproduction procedures. Furthermore, differently from carbon-11, it allows the distribution of [^{18}F]-labeled compounds within a few hours drive from the production site, thus attracting commercial interest.

[^{18}F]-fluorination strategies cover an important role in radiochemistry, and they are often used for the translation of [^{11}C]-radiotracers to [^{18}F]-analogs to overcome the main constraints in the manipulation of carbon-11 compounds. However, the approaches for incorporating a fluorine-18 in a molecule remain much more limited than those of carbon-11, due to the chemical nature of this element. The main synthetic strategies behind [^{18}F]-labeling can be classified into “direct fluorination,” a one-step reaction, and “indirect fluorination,” which requires a multistep synthetic procedure. The direct introduction of [^{18}F]fluorine into a molecule is often accomplished through a nucleophilic substitution reaction using no-carrier-added in-target-produced [^{18}F]fluoride, an enhancer for [^{18}F]nucleophilicity

(Kryptofix-222) and a precursor with a good leaving group (triflate, mesylate, tosylate). After labeling, the crude reaction mixtures have to be purified by semipreparative HPLC or solid-phase extraction (SPE) techniques, to provide pure radiopharmaceuticals as sterile, pyrogen-free isotonic solutions suitable for intravenous injection. This simple method has the advantage to be easily adapted to many commercially available automated synthesizers, and due to the large availability of suitable precursors, it is routinely used to efficiently produce some of the most important [^{18}F]-radiotracers (some examples are [^{18}F]FDG, [^{18}F]FLT, [^{18}F]fallypride, and [^{18}F]FAZA). In addition, nucleophilic substitution may also be applied for the insertion of fluorine-18 into aromatic systems, such as for [^{18}F]flumazenil or [^{18}F]FDOPA, with high specific activity [94, 95].

Electrophilic fluorination using [^{18}F]F₂, produced by cyclotron irradiation, is an alternative direct strategy that was successfully developed in the early years of PET radiochemistry. Few clinical radiopharmaceuticals still rely on regioselective aromatic electrophilic [^{18}F]fluorination synthesis, for example [^{18}F]FDOPA [96]. Nevertheless, the general use of this labeling method is hampered by the low specific activity of the final [^{18}F]labeled compounds and a complicated high-pressure gas target, imposing a severe limitation for the development of targeted brain imaging agents.

When direct substitutions are not accessible, a common alternative is the “indirect [^{18}F]labeling via prosthetic groups.” This process consists in the synthesis of a secondary small labeled precursor, typically a [^{18}F]fluoro-alkylating agent, which can be easily introduced into the molecule of interest. Prosthetic groups are versatile and they can react with the amine, amide, carboxylic, and phenolic groups of the “cold” precursors to obtain the [^{18}F]fluoro-alkylated radiotracers [97]. Among the most popular alkylating agents, [^{18}F]fluoro-methyl/ethyl/propyl bromine and tosylate have been used for the production of radiopharmaceuticals, such as [^{18}F]fluoromethyl and ethyl choline, [^{18}F]FET, and [^{18}F]FP-CIT [98, 99].

4.3.4.3 Iodine-123

Iodine-123 represents the main alternative to PET emitters for the design of target-specific radiotracers, thanks to its low energetic gamma emission and a half-life of 13.2 h suitable for SPECT imaging. Due to its easy and well-established chemistry, which allows its incorporation into a wide variety of molecules, iodine-123 holds a major interest in the development of radiotracers for nearly three decades. Furthermore, the advantageous half-life and the very high purity of iodine-123 starting radioactive material (which lead to high specific activity radiotracers) allow industrial production and delivery for hospital use over long distances although the highest radionuclidic purity I-123 requires a high-energy cyclotron.

Numerous small molecules have been studied as probes for brain receptors and enzymes, and iodine-123 has become the SPECT radionuclide of choice for development of conventional radiopharmaceuticals for brain imaging. However, iodine has a considerable dimension if compared with PET radionuclides (steric hindrance is similar to a methyl group), and therefore radiolabeling, depending on where iodine is incorporated, could deeply modify the kinetic and target affinity of the molecule. For instance, [^{123}I]- β -CIT, tropane-labeled derivative with relevant use in clinical setting, suffers serious drawbacks. It

shows a low target selectivity, binding equally to both dopamine and serotonin transporters (DAT and SERT), and it has a slow kinetics which impose the operators to perform the imaging sessions within 24 h after bolus injection. [^{123}I]IPT and [^{123}I]PE2I have chemical structures similar to [^{123}I]- β -CIT, but they show much faster kinetics and improved selectivity for DAT versus SERT [100–102] (Fig. 4.3).

Radioiodination is the process that introduces a radioactive iodine into a chemical structure. Due to its relatively long half-life, the timing of the labeling reaction is only a minor limitation, and the production strategy focuses mainly on the one-step preparation and purification with high yield. Direct labeling is by far the most common method, based on the radionuclide insertion in an aromatic ring or vinylic groups using N-chloroamides. These reagents generate iodine cations from sodium iodide, which promptly react with activated groups through electrophilic substitution of good leaving groups. Radioiodination by demetallation, a commonly applied procedure, allows a regioselective labeling in almost a quantitative yield, preventing the formation of iodine side products that may be difficult to remove [103]. Some examples of the most important iodine-123 radiopharmaceutical for brain imaging are [^{123}I]-FP-CIT, [^{123}I]- β -CIT, [^{123}I]iomazenil, [^{123}I]IBZM, and [^{123}I]epidepride.

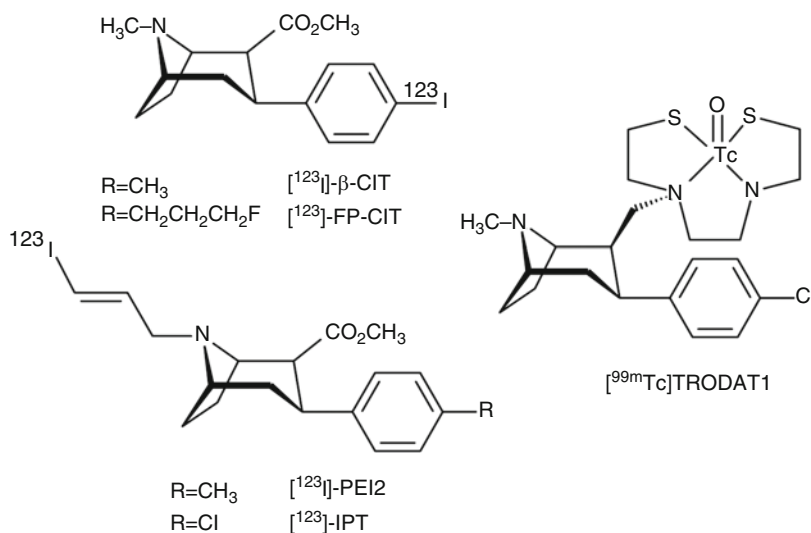


Fig. 4.3 Chemical structures for SPECT tropane derivatives [^{123}I]- β -CIT, [^{123}I]-FP-CIT, [^{123}I]-IPT, [^{123}I]-PE2I, and TRODAT1

Unfortunately, the production costs, especially those related to iodine-123 precursor, are still relatively high in comparison with other radionuclides, thus limiting the future interest for the development of new iodine-based radiotracer.

4.3.4.4 Technetium-99m

As an alternative to carbon-11, fluorine-18, and iodine-123, technetium-99m could be more widely used for the preparation of SPECT neuro-imaging agents due to its relative low cost and easy availability. Different from the former radionuclides where a covalent attachment is required for radiolabeling, technetium-99m has to be incorporated through a chelation because of its metal nature. In the traditional labeling method, called the “pendant approach,” the radiometal is stabilized through an external chelating system, covalently bonded to the bioactive molecule. Several CNS technetium imaging agents have been labeled using the pendant approach, which unfortunately often produces molecules suffering from metal core hindrance on receptor interaction and an overall excessive dimension which does not facilitate BBB crossing. This resulted in the development of very few technetium-99m radiotracers with suitable imaging properties and in only one agent, [^{99m}Tc]TRODAT-1, currently approved and used in clinical settings in Taiwan (Fig. 4.3) [64, 65, 104, 105].

To overcome constraints due to the presence of a voluminous external complex, technetium-99m “core” may be ideally incorporated within the chemical structure of the radiotracer. This strategy is defined as an “integrated approach,” and the resulting molecule/chelated system has to both stabilize the radiometal and demonstrate affinity for the target. However, the insertion of such a foreign element causes dramatic modifications in the chemical properties of small molecules. This, together with the existent severe restrictions for the design of a brain imaging radiotracer, still represents an outstanding challenge for radiochemists and successful examples are rare. Researchers have been working for a long time on the development of new technetium-99m-based radiotracers, with major focus coordination chemistry (technetium can exist in

different oxidation states) and labeling strategies, which play a major role for improving the critical issue of brain uptake. Many efforts have been made with the inert, compact, and versatile *fac*-[^{99m}Tc]tricarbonyl core after the year 2000 [106]. The interest for this new labeling approach was promoted by the development of a single vial freeze-dried kit for the preparation of [$^{99m}\text{Tc}(\text{OH}_2)_3(\text{CO})_3$] $^+$, suitable for routine procedures in conventional Nuclear Medicine [107]. Despite the fact that penetration of the normal BBB remains a challenge for most of [^{99m}Tc]tricarbonyl radiotracers, not so much because of log P but due to molecular size, a few recent examples provided encouraging evidence that development of a promising brain imaging agent may be feasible [108, 109].

Certainly, to reduce the costs of brain receptor imaging, it would be desirable to have more technetium-based pharmaceuticals, rather than PET or iodine radiotracers. This, as mentioned above, would further allow a vast worldwide access to these brain diagnostic techniques, especially in developing countries where access to cyclotrons is still limited.

4.3.4.5 The Physical Half Life of a Radionuclide and the Biological Half Life of a Radiotracer

The half-life of the radionuclide in a radiotracer should correlate with the kinetic of the process to investigate. In other words, the radiotracer, after injection, should cross the BBB and interact quantitatively with the target, in a time frame that has to be consistent with the radionuclide half-life. Iodine-123 and technetium-99m allow extended imaging protocols due to their relatively long half-lives, and thus they may be employed to investigate slow kinetic processes. On the other hand, positron emitters with short half-lives, such as carbon-11 and fluorine-18, impose faster kinetics in order to gain maximum target uptake before a substantial radioactive decay occurs. Very short-lived nuclides, such as oxygen-15, are appropriate for preparing simple molecules with rapid kinetic to be used as perfusion agents as [^{15}O]water, [^{15}O]O $_2$, or [^{15}O]CO. An

optimized correlation between the half-life of a radionuclide and the process to investigate is also important in order to avoid unnecessary radiation burden to patients when long-lived radionuclide is used.

All these considerations are based on the assumption that a radiotracer is stable enough to reach the target intact. However, most radiotracers are significantly metabolized *in vivo*, and the assessment of the radiotracer metabolism, as well as the fate of its metabolites, is crucial for designing a successful application for diagnostic purposes.

Immediately after administration, radiotracers are susceptible to BBB selection and processing by an array of enzymes in the blood and other tissues. This may dramatically decrease the amount of labeled molecules and have a substantial impact on radiotracer distribution and target-to-nontarget ratio. The rate of metabolism determines the duration and efficacy of a radiotracer, also known as the biological half-life. It is key to note that external imaging detects the emitted photons, but contains no information on the chemical structure.

The ability of a molecule to cross the BBB depends on the balance between its passive transfer and its exit due to efflux pumps, such as P-glycoprotein (P-gp) and multidrug resistance-associated proteins [110, 111]. It is generally assumed that passive entry is facilitated for molecules with low weight (<500 Da) and moderate lipophilicity (typically measured octanol/water partition coefficient-logP) [112]. Hydrophilic or charged compounds are excluded by BBB, with some important exceptions for nutrients, such as choline or amino acids that can pass through active transporters. On the other hand, an excessive lipophilicity determines an increased binding to plasma proteins and nonspecific interaction with brain fats and proteins.

If an adequate brain entry is a key aspect for SPECT and PET radiotracers, *in vivo* stability is also crucial and it has to be also accurately evaluated. Drug metabolism, also known as xenobiotic metabolism, involves the biochemical modification of substances such as pharmaceuticals or radiotracers. Hydroxylation, N-demethylation,

and oxidation are some of the most common enzyme-mediated reactions that may occur *in vivo* producing less lipophilic radioactive metabolites, which should then be excreted. The nature and degree for the metabolism of a radiolabeled compound are dependent upon its molecular structure, and especially for low-density-easily saturated sites, it can have a significant influence on the radioactive distribution and, eventually, on the reliability of the image scanning. In fact, tomographic systems detect the radionuclide signal regardless of its origin from the intact radiotracer, from a radiolabeled metabolic fragment or from the free radionuclide. This may result in a misleading acquired image due to the presence of several radioactive molecules. Since some of them are correlated to the parent chemical structure and compete for target occupancy, a biomathematical analysis is required for the quantization of regional brain radioactivity uptake [113].

Other common metabolic pathways for radiotracers involve the removal of [^{11}C]methyl or [^{18}F]fluoroethyl substituents from radiotracers or, in case of halogenated radiotracers, direct defluorination or deiodination [114]. Radiodefluorination is a relevant issue for [^{18}F]-radiotracers leading to the release of [^{18}F]fluoride *in vivo*, which is then accumulated in bone tissue. In particular for brain imaging, an undesired accumulation may produce PET images where tissue uptake near the skull is more difficult to interpret [115].

Radiotracer metabolism is not easy to predict, and although it remains an undesired effect, it does not always alter the final quality of the image. If radiotracer processing occurs outside of the CNS, all the resulting radioactive hydrophilic metabolites will be excluded by the BBB. [^{11}C]methyl-radiotracers, for example, are susceptible to demethylation leading to small polar radiometabolites (e.g., [^{11}C]formaldehyde) that don't interfere with brain uptake.

These considerations suggest that an effective molecular design has to take into account in order to prevent the formation of potential radioactive derivatives that may have negative consequences [114–116] for brain imaging. Several examples are reported in literature, where the chosen point of insertion of a radionuclide in the molecule

chemical structure will elicit radiotracer degradation to fragments that don't interact with the desired target or with lower permeability for BBB [117]. In the preparation of [^{11}C]volinanserin, a radiotracer with affinity for $5\text{HT}_{2\text{A}}$ receptors, carbon-11 can be incorporated by methylation at the hydroxyl groups in positions 2 or 3 of the aromatic ring [118]. Both radiolabeled molecules are susceptible of enzymatic demethylation at position 3, but while the first results in the formation of lipophilic radioactive derivative that are able to cross the BBB and compete for target occupancy, the second is prone to produce a hydrophilic metabolite that is excluded from the brain (Fig. 4.4) [119, 120]. Labeling on position 3 is, in this case, the preferred choice.

The importance of inserting a radioisotope in different chemical groups and its influence on the radioactivity distribution in the brain have been also cleverly demonstrated with the $5\text{-HT}_{1\text{A}}$ radioligand [^{11}C]WAY-100635 [121]. In this example, chemical structure enables carbon-11 insertion either in a methoxy group or in a carbonyl position (Fig. 4.4). Carbon-11 was first introduced through methylation, but since primary metabolic pathway in humans occurs through hydrolysis of the amide bond, [^{11}C]WAY-100635 metabolism resulted in the formation of

an active metabolite with affinity for $5\text{-HT}_{1\text{A}}$ binding and for $\alpha 1$ -adrenergic receptors in the brain. In this case, metabolites were interfering with PET measurements. Only subsequently labeling position was modified into a carbonyl motif, thus avoiding [^{11}C]WAY-100635-labeled metabolites entering the brain and providing improved high PET signal and advantages in biomathematical modeling [122, 123].

A different approach for reducing the presence of radioactive metabolites is represented by the use of inhibitors for the enzyme responsible for the radiotracer major metabolic pathway. Carbidopa, for instance, a known inhibitor of aromatic amino acid decarboxylase, is clinically used for increasing total and specific brain tomographic activity per unit dose of [^{18}F]FDOPA [124]. Other inhibitors have been recently successfully tested although, due to the complexity of their metabolic pathways, the molecular design of a radiotracer remains the main measure for limiting the undesired metabolism [125, 126].

4.3.4.6 Specific Activity

Specific activity (SA) is one of the most important parameters (especially for radiotracers for low-density sites) to assess during radiotracer and radiopharmaceutical development, and it is

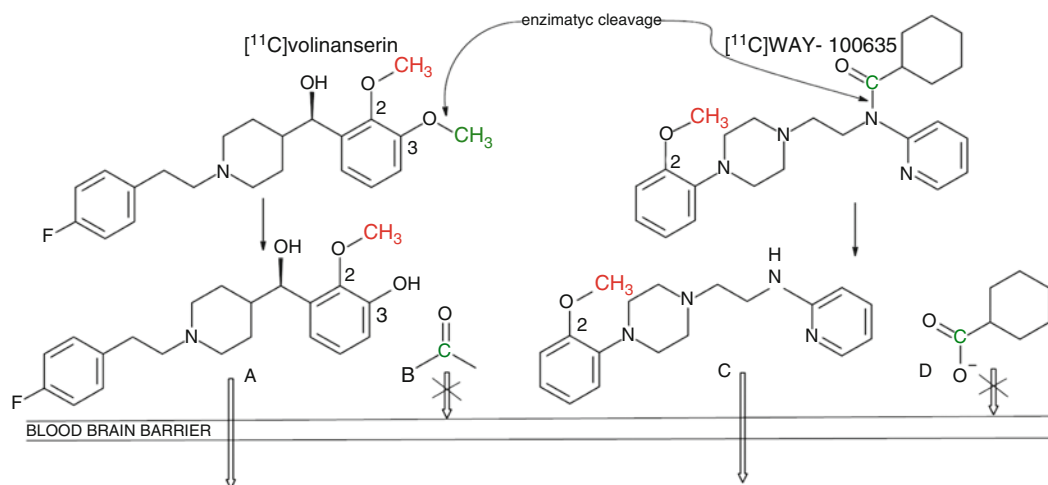


Fig. 4.4 [^{11}C]Volinanserin and [^{11}C]WAY-110635: choice of position of radiolabel as function of metabolism. Labeling with carbon-11 in red position produces for both tracers radiometabolites that interfere with the

brain and specific receptor uptake (a and c). Labeling in green position produces “cold” metabolites that enter the brain, while radiometabolites are excluded by BBB (b and d)

defined as the amount of radioactivity per unit mass of a radiolabeled compound. This mass includes the mass of the radioactive product and the mass of its nonradioactive counterpart, determined typically by spectroscopic or electrochemical methods. SA is measured in Ci/ μ mol or GBq/ μ mol units, and for short-lived radionuclides, the time that the measurement of SA is key.

SA of a diagnostic agent must be congruent with the “radiotracer principle,” where the overall mass of the administered tracer should be able to trace a process without inducing an unwanted biological response. This means that, for imaging studies, the amount of radioactivity to be administered has to be sufficiently high to allow the acquisition of good-quality images and low enough to prevent detector saturation and an excessive patient radiation exposure. At the same time, the radiopharmaceutical preparation, including all components, has to present a safe profile with low toxicity.

Every radioisotope or radioactive molecule is characterized by a specific SA; however, its evaluation may not be critical for all application with radiotracers. This is the case for true tracers (such as [^{11}C]acetate and [^{11}C]methionine) since the nonradioactive endogenous compounds are normally found in μM or higher concentrations in the organism and for high-capacity system radiotracers, such as agents for measuring regional cerebral blood flow, hypoxia, and glucose metabolism (e.g., [^{15}O]water or [^{18}F]FDG). In fact, due to the large capacity of these biological systems, radioactivity distribution is not significantly affected by the presence of relatively high concentration of “cold” molecules with identical or similar kinetic properties; thus, the acquired images may correctly represent organ functionalities or metabolic activities.

On the contrary, SA becomes crucial when the radioactive and nonradioactive molecules contained in the radiotracer batch may produce undesired pharmacodynamics–toxicological effects as well as target occupancy or saturation. Specific imaging applications, such as the study of toxic molecules behavior or the visualization of low-density receptors and enzymes in the brain, have to pay particular attention to SA. The

required SA for a given radiotracer depends on the concentration of the interested low-capacity easily saturated sites, especially for receptors, which are typically in the range of 0.1–50 nM.

This has been recently demonstrated for [^{11}C]raclopride in an animal model study, where in case of a low specific activity radiotracer, significant receptor occupancy occurred by unlabeled dopamine, thus altering dramatically the target uptake [127].

Several factors may have a substantial impact on the final radiotracer SA. Ideally, the radionuclide employed for radiotracer development should be carrier-free, which means that it does not have to be contaminated by either stable radionuclides or other isotopes of the same element. Technetium-99m and iodine-123 closely match this property [128]. Furthermore, labeling should be carried out with a minimal amount of cold precursor, thus reducing the possibility of competition for target binding. Unfortunately, many fast reaction and high-labeling yields are achieved using a large excess of reagents; thus, the final preparation may contain biologically active impurities, such as residual precursor or side products from the labeling reaction, which were not removed by purification.

SA parameter is considered to be critical for many PET radiotracers. Although theoretical SA values for the most common used positron emitters are very high (340 TBq/ μ mol for carbon-11, 63 TBq/ μ mol for fluorine-18), these experimental values are usually very far from those obtained after radiotracer synthesis. The required SA for radiotracers that have to interact with receptors are generally in the range of 37–370 GBq/ μ mole, while lower SA may be adequate for enzyme-mediated molecular imaging studies. This decrease in SA is generally due to a dilution process with nonradioactive isotope, usually occurring in the radionuclide manipulation for the radiotracer preparation (from radionuclide production to labeling reaction). In fact, final specific activities of [^{18}F]- and [^{11}C]labeled radiotracers are extremely dependent on the production methods as well as the technologies, reagents, equipment, and procedures employed. Minimal alterations of experimental conditions can lead to

considerable effects on SA, especially when high values are required. For this reason, the same radiotracer may be produced with different values of SA depending on the production sites, and this produces the unpleasant consequence having different image quality from one PET center to another.

Therefore, considering the increasing expansion of PET radiopharmaceuticals and the influence of SA for neuroimaging studies, the establishment of harmonized protocols for each radiotracer production and use are certainly desirable. Furthermore, a definition of SA values for brain imaging radiotracers may also facilitate the approval of new radiopharmaceuticals for human use through regulatory authorities [129].

4.4 SPECT/PET Brain Imaging Radiopharmaceuticals

Radiopharmaceuticals and radiotracers for the investigation of neurological disorders with SPECT and PET have found many diagnostic applications. A large number of radiolabeled molecules have been developed, but only a few reached clinical state in Nuclear Medicine. After administration, typically by intravenous injection, the regional uptake and distribution of radioactivity are evaluated using tomographic systems. A selection of the most important radiopharmaceuticals routinely employed in clinical practice are reported in Table 4.2 and described as follows.

4.4.1 Flow and Perfusion

Radiopharmaceuticals applied in the evaluation of rCBF are transported by diffusion from the arterial vascular compartment into the normal brain tissue compartment. The distribution pattern depends on the interaction with brain tissue. Xenon-133 and krypton-81m for SPECT and [^{15}O]oxygen and [^{15}O]water for PET are freely diffusible and not trapped; thus, rCBF is evaluated by determination of the clearance rate of the tracer after a brief uptake period. [^{15}O]CO (carbon

monoxide) is assumed to have a different mechanism, accumulating selectively in red blood cells when inhaled in tracer quantities. [^{15}O]CO labels hemoglobin *in vivo* forming carboxyhemoglobin, thus making possible the delineation of the cerebral vascular blood pool [130].

[$^{99\text{m}}\text{Tc}$]HMPAO and [$^{99\text{m}}\text{Tc}$]ECD are commonly used in clinical routine, and their use allows to monitor the changes of rCBF in various neurological diseases. Both complexes penetrate the intact BBB and their distribution correlates with rCBF. [$^{99\text{m}}\text{Tc}$]HMPAO retention is due to its chemical instability. After BBB penetration, technetium-99m dissociates forming complexes with intracellular proteins, rendering it unable to re-diffuse through the BBB and thus trapping radioactivity in the brain. The redox status of the tissue and the concentration of glutathione have been identified as critical parameters for this conversion [131]. [$^{99\text{m}}\text{Tc}$]ECD brain uptake is otherwise attributed to the enzymatic hydrolysis of an ester group and the formation of a hydrophilic metabolite, which cannot diffuse back out across the BBB, thus allowing SPECT imaging [132].

4.4.2 Tumor Metabolism

Despite the fact that [^{18}F]FDG is the “working horse” for diagnostic imaging studies in neuro-oncology (over 90% are made with [^{18}F]FDG worldwide), other radiopharmaceuticals more tumor specific than [^{18}F]FDG are available. Among these, radiolabeled amino acid and choline derivatives (true tracers or analog tracers) are routinely employed for the diagnoses of primary and metastatic brain tumors. [^{11}C]methionine as well as other PET-labeled aromatic amino acid analogs, such as [^{18}F]FET and [^{18}F]FDOPA [133], are able to delineate the increased rates of amino acid transport by many tumor cells. [^{123}I]IMT, a gamma-emitting radiopharmaceutical, represents the SPECT clinical alternative due to similar *in vivo* behavior. Abnormal choline metabolism is another metabolic hallmark associated with oncogenesis and tumor progression. Choline is a cellular membrane phospholipid precursor that can be phosphorylated by the enzyme choline

kinase and further incorporated into phosphatidylcholine, a major phospholipid of all membranes. Radiolabeled choline derivatives are mainly used to diagnose prostate cancer; however, they have an established use for brain imaging [134].

[¹⁸F]FLT, a nucleoside-labeled analog, is also employed as radiopharmaceutical in oncology and neuro-oncology. It is intracellularly phosphorylated by the thymidine kinase 1 (TK1), and the corresponding nucleoside monophosphate is not further metabolized. Since TK1 has a higher activity in proliferating tumor cells with respect to normal tissue, [¹⁸F]FLT uptake represents an *in vivo* biomarker of proliferation activity [135].

²⁰¹Tl and [^{99m}Tc]MIBI are commonly used to image myocardial perfusion. Thallium-201 uptake in brain tumors is a result of a combination of factors, such as alterations in the BBB variability in the expression of the Na⁺/K⁺ pump and blood flow. [^{99m}Tc]MIBI accumulation depends on altered P-glycoprotein function on BBB and passive transport into tumor tissue mitochondria. Mainly in the past, cardiac radiopharmaceuticals have been widely used as neuro-oncological radiotracers, being able to be selectively incorporated by active proliferating cells comparing with normal brain tissue [133].

4.4.3 Dopaminergic System

Dopaminergic neurotransmission has a central role in many brain functions. The presynaptic nigrostriatal projection is the main location of the pathologic process in Parkinson's disease, and therefore, the assessment of disturbed dopamine synthesis is the main target for clinical studies. For measuring dopamine synthesis, the main radiopharmaceutical used is [¹⁸F]FDOPA [89]. Postsynaptic receptors may also be involved in neurodegenerative disorders. The dopamine receptor family is composed by five subtypes, but the most studied is D2. N-[¹¹C]methylspiperone was one of the first radiotracer employed for the study of dopamine receptor reserve, but it also has affinity for 5-HT₂ receptors. The gold standard PET tracer for D2 receptors is [¹¹C]raclopride at

present, which has allowed the analysis of central D2 receptors (although it also binds D3 receptors) [136]. For SPECT, [¹²³I]IBZN is the most widely used D2 receptor tracer, while for PET, [¹⁸F]fallypride is especially suitable for investigation of extrastriatal D2 receptors.

The neural dopamine transporter (DAT) is a membrane-bound presynaptically located protein that regulates the concentration of dopamine in nerve terminals. The evaluation of the reuptake sites provides a measure of the density of dopaminergic nerve terminals. Several compounds have been shown to be antagonists of the monoamine reuptake system, and many tropane derivatives labeled with single-photon emitters are currently in use.

4.4.4 Other Neurotransmitting Systems

Acetylcholine is a neurotransmitter at cholinergic synapses that mediate functions on nicotinic and muscarinic receptors. Nicotinic receptors have been implied in many psychiatric and neurologic diseases, including depression and cognitive and memory disorders. 2-[¹⁸F]F-A85380 is a potent and selective agonist suitable for imaging the nicotinic subtype $\alpha_4\beta_2$ nACh receptors [54]. The muscarinic reserve, which is the dominant postsynaptic cholinergic receptor in the brain, has been targeted by many tracers for SPECT and PET. However, due to the lack of selectivity for the receptors subtypes, there are not radiopharmaceuticals in clinical state at present. On the contrary, the quantitative measurement of the acetylcholinesterase activity in the brain by radiolabeled acetylcholine analogs, such as [¹¹C]MP4A, has found broader application [52].

GABA is the most important inhibitory neurotransmitter which is altered in epilepsy, anxiety, and other psychiatric disorders. The radiopharmaceutical most widely used for central benzodiazepine-binding sites is the antagonist flumazenil labeled with carbon-11 or iodine-123 [43]. Another binding site for benzodiazepine in the brain is the peripheral benzodiazepine receptor (PBR), which is located in the mitochondrial

and nuclear subcellular fraction. The most frequently used radiopharmaceutical as the ligand for the PBRs is [^{11}C]PK11195, which is a valuable tool for imaging the activation of microglia *in vivo* [39].

4.4.5 Amyloid-Binding Ligands

The amyloid ligands in clinical use are the thioflavin T-derived [^{11}C]-Pittsburgh compound B (PIB) and [^{18}F]flutemetamol, [^{18}F]florbetaben, and [^{18}F]florbetapir [72]. [^{18}F]labeled analogs are approved by the Food and Drugs Administration (FDA) and European Medicines Agency (EMA) for the clinical evaluation of cognitive deficits in patients and available in the market.

4.4.6 Cisternography

Cisternography with [^{111}In]DTPA is indicated as an imaging agent to study the flow of cerebrospinal fluid (CSF) in the brain, to diagnose abnormalities in CSF circulation, to assess and help localize the site of CSF leakage, and to test the patency of or localize blocks in CSF shunts.

4.5 Future Brain Imaging Directions

An area of great excitement is the detection of neurotransmitter system fluctuations for the study of drug abuse or behavioral addictions. Addiction is the habitual compulsion to use a substance (i.e., alcohol, opiate, nicotine) or to engage in an activity without much regard for its detrimental effects on a person's physical, mental, financial, and social well-being (i.e., gambling, food, work, love). Brain imaging is a valuable tool that may help to establish addiction as a "disease," leading to the characterization of the involved neural circuitry in intoxication, withdrawal, and abstinence. Furthermore, the evidence of an altered neurotransmitter system may lead to the identification of a potential target for pharmacological treatments.

A key discovery has been the blunted striatal dopaminergic activity, at presynaptic and postsynaptic levels, in a variety of addictions [137]. Dopaminergic neurotransmission was found altered after repeated drug exposure, and the measurement of dopamine release has been described with SPECT and PET using the available radiopharmaceutical for dopamine receptors and DAT transporters.

While studies of addiction have been well established for the dopamine system, extension to other systems has been limited by the lack of suitable radiopharmaceuticals. However, within the past 10 years, and in particular in the last few years, promising tools have been made available for imaging fluctuations in 5-HT, endogenous opioids, GABA, and glutamate [73].

Conclusions

There are two main areas of research using PET-CT and PET-MRI imaging of neurological disease, namely, increasing the understanding of human biology and improving patient care through diagnostic imaging. Major advances have been made in understanding the complicated interactions in the brain. Although genetic and environmental factors are contributors to abnormalities, neurological and psychiatric diseases are not correlated with a single genetic abnormality. *Ex vivo* measurements using proteomic analysis of biopsy samples are most difficult in the brain giving external nuclear imaging an added value. As a result, measuring the changes in the biochemistry of the protein expression products and their neurotransmitters has become the province of nuclear imaging. The addition of anatomical instruments to PET and SPECT has made a tremendous difference in co-registering the small anatomical structures with the emitted radioactivity. SPECT/CT and SPECT/MRI have benefited more than PET/CT and PET/MRI given the lower resolution of SPECT [138].

The understanding of addiction, for example, has been enhanced by PET studies of receptor occupancy and synaptic neurotransmitter changes, especially for the dopamine system [139, 140].

Improving patient care through diagnostic imaging has been more difficult given the complexity of most diseases. The restrictions of patient tolerance and absorbed radiation dose have limited external imaging to one or two studies using probes for different biochemistry. The major achievements have been in monitoring general disease control points, mostly in tumors, or serving as a biomarker for potential drugs by measuring occupancy and changes in biochemistry as a function of disease progression or treatment. A case in point is the plaque imaging agents that are clearly biomarkers for the presence of plaque, but do not impact uniquely on diagnosing Alzheimer's disease. Overall, the combination imaging devices have increased the usefulness of radiopharmaceuticals by leading to more precise information for the location and the amount of radiopharmaceutical in specific areas of the brain.

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Glossary

Radionuclide A radioactive nuclide is an unstable form of a chemical element that decays by emitting particles, gamma radiation, or X-radiation.

Radioisotope An unstable isotope of an element that decays by emitting particles, gamma radiation, or X-radiation. The term, "radioisotopes," is limited to unstable atoms of the same element.

Radioindicator First used by Hevesy to describe the use of physiologic elements. It was used later to describe indicator dilution studies using radionuclides in cardiology.

Radiotracer This should be reserved for those radiolabeled compounds that contain a radioisotope of a stable element and not a radionuclide. The latter requires extensive validation to demonstrate that it is a radiotracer.

Radiopharmaceutical The term for a radiolabeled compound after approval or registration

by a government agency. This nomenclature reflects the similar requirements for identity, purity, and safety that are required of pharmaceuticals.

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