RADIOTRACERS FOR PET AND SPECT STUDIES OF NEUROTRANSMITTER SYSTEMS

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Abstract: The study of neurotransmitter systems is one of the major thrusts in emission tomography today. The current generation of PET and SPECT radiotracers examines neurotransmitter properties from a number of different perspectives including their pre and post synaptic sites and the activity of the enzymes which regulate their concentration. Although the dopamine system has been the most extensively investigated, other neurotransmitter systems including the acetylcholine muscarine, serotonin, benzodiazepine, opiate, NMDA and others are also under intensive development. Enzymes involved in the synthesis and regulation of neurotransmitter concentration, for example monoamine oxidase and amino acid decarboxylase have also been probed in vivo. Medical applications range from the study of normal function and the characterization of neurotransmitter activity in neurological and psychiatric diseases and in heart disease and cancer to the study of the binding of therapeutic drugs and substances of abuse.

This chapter will provide an overview of the current generation of radiotracers for PET and SPECT studies of neurotransmitter systems including radiotracer design, synthesis localization mechanisms and applications in emission tomography.

INTRODUCTION

Single photon emission computed tomography (SPECT) and positron emission tomography (PET) are non-invasive physical probes for imaging the brain and other organs of the human body. When coupled with highly selective radiotracers, these imaging methods allow the examination of biochemical transformations by detecting the regional and temporal distribution of labeled compounds. With high specific activity tracers, PET and SPECT can be used to probe biochemical processes at true tracer levels where the chemical mass associated with the labeled compound is far below that which would elicit a pharmacological or a toxic reaction.

The study of neurotransmitter systems is one of the major thrusts in emission tomography today and the current generation of PET and SPECT radiotracers allows the examination of neurotransmitter properties from a number of different perspectives including their pre and post synaptic sites and the activity of the enzymes which regulate their concentration. Although a major focus has been on the brain, the study of neurotransmitter activity in the heart and in some tumors has also been addressed.

This chapter will provide an overview of the current generation of radiotracers for PET and SPECT studies of neurotransmitter systems. For a more comprehensive treatment of this subject, the reader is referred to recent chapters and review articles (1,2,3,4,5).

The Technology

The technology required to apply PET and SPECT to problems in biology and medicine is rapidly advancing. However, there are two fundamentally important technological considerations in implementing PET and SPECT methods. These are (1) the need for a cyclotron for PET radiotracer development and application and (2) the characteristics of the current generation of PET and SPECT imaging devices and its impact of this on the design of the radiotracer and on quantitation.

Cyclotrons: Research in the PET field is dominated by the short half-life to the commonly used nuclides shown in Table 1.

TABLE 1
POSITRON EMITTER AND SINGLE PHOTON EMITTER RADIONUCLIDES AND PRECURSORS FOR NEUROTRANSMITTER RADIOLIGAND SYNTHESIS

Nuclide	Half-Life	Decay	Precursors
PET			
Carbon-11	20.4 Min	β^+(0.960 MeV)	$^{11}CO_2$, $H^{11}CN$
Fluorine-18	109.8 Min	β^+(0.635 Mev)	$^{18}F^-$
SPECT			
Iodine-123	13.3 Hr	γ(0.159 MeV)	$^{123}I^+$

While it is possible to carry out certain PET studies with generator produced PET nuclides, neurotransmitter receptor studies require organic compounds typically labeled with carbon-11 or fluorine-18. This, in turn, requires the availability of an on-site cyclotron or other accelerator (6) to produce the short lived nuclides. Although it would be possible to produce and distribute F-18 labeled radiotracers from a regional center to surrounding institutions between a 2 and 3 hour shipping radius given the 110 minute half-life of fluorine-18, no such center exists at the present time. Thus at the present time, research and application with these nuclides requires the presence of an on-site cyclotron, a chemistry laboratory and a PET instrument appropriately staffed to carried out basic and applied research.

Imaging Instrumentation: PET and SPECT imaging instrumentation is rapidly advancing with significant effort being expended on improving resolution and sensitivity and the quantitative accuracy of the measurements (7). The major differences between PET and SPECT imaging instrumentation which influence the development of their respective radiotracers is the relative sensitivity of detection and quantitation accuracy. The sensitivity of a SPECT instrument is much less than that of a PET instrument of similar resolution. Therefore an important property of a SPECT radiotracer, in addition to its selectivity for a particular neurotransmitter system, is that it have a sufficiently long residence time in tissue so that appropriate aquisition times can be used to maximize the statistical quality of the image.

Chemistry (see Table 1 for PET and SPECT nuclides for labeling neurotransmitter ligands and available chemical forms): PET and SPECT share many common goals in terms of their applications to the study of neurotransmitter systems. However, significant differences in the chemical properties of the PET and SPECT radionuclides dominate the spectrum of their respective radiotracers. One relatively common feature of the ligands use for PET and SPECT studies of neurotransmitter systems in the brain is that they are relatively small (MW<500) organic molecules. Labeling them without changing the properties of the parent molecule or using the label to enhance the properties of the parent molecule is therefore a requirement. The "organic" PET nuclides form covalent bonds and thus can usually be incorporated into organic molecules without altering the properties of the parent molecule. However, their synthetic incorporation into organic molecules, especially in high specific activity requires the development of rapid methods including sophisticated purification protocols and quality control of the resulting labeled compounds. Several chapters and monographs provide a detailed description of the problems involved (1,2,8,9,10).

Thus far SPECT nuclides for probing neurotransmitter systems is limited to radioiodine, usually iodine-123, because radioiodine forms covalent bonds to carbon. Iodine is a relatively large atom and its attachment to small organic molecules without altering the properties of the parent molecule represents a particular challenge. Interestingly, there are examples where the attachment of iodine actually enhances the selectivity of the parent molecule for a particular neurotransmitter system as well as examples where iodine substitution results in a molecules with biological properties which bear little resemblance to the parent molecule. While generator produced technetium-99m is the ideal SPECT nuclide in terms of availability, it possesses metallic properties and its incorporation into a small organic molecule generally masks the properties of the parent molecule.

For neurotransmitter studies high specific activity where the dilution of radioisotope by the naturally occurring element can be in the range of factors of 100 to 10,000, is required in order to avoid saturation and measureable physiological reactions to the labeled compound.

Radiotracer Design: Radiotracers for probing a specfic neurotransmitter systems must display appropriate biological behavior. This includes high uptake in the region of interest, a pharmacological profile and regional distribution consistent with binding to a specific neurotransmitter system, an absence of labeled metabolites which also localize in the region of interest and kinetic behavior which allows the distinction of its interaction with the binding site from its delivery to this site. The literature of biochemistry and pharmacology is an essential resource in guiding the design of the radiotracer. Both in vitro and in vivo autoradiography of tritium labeled ligands are of particular value and autoradiographic studies reporting changes in neurotransmitter systems in post mortem human brain can serve to stimulate the examination of the neurochemical changes in a number of disease states with PET and SPECT (11).

Once a PET or SPECT labeled tracer has been developed, mechanistic studies can also be carried out in animals in vivo where non-invasive imaging can be used to assess the absolute uptake, the regional distribution, clearance rate, the pharmacological profile, the stereoselectivity and kinetic isotope effects.

PET and SPECT Radiotracers: Examples

The first human studies of neurotransmitter systems examined the binding of radiotracers to the acetylcholine-muscarine system and the dopamine D2 systems with SPECT and PET respectively (12,13). Other neurotransmitter systems including the serotonin, benzodiazepine, opiate, NMDA and others are also under intensive

development. Enzymes involved in the synthesis and regulation of neurotransmitter concentration, for example monoamine oxidase has also been probed in vivo (14,15).

Today, the dopamine system and the cholinergic systems are still the most extensively investigated both from the standpoint radioligand development and medical application and progress in the study of these two systems with PET and SPECT is summarized in the following sections:

The Dopamine System: Interest in the dopamine system stems from its association with Parkinson's disease, schizophrenia and abuse of the psychostimulants such as cocaine and amphetamine (16,17,18). Radiotracer development has included ligands which are selective for the post-synaptic receptors (D1 and D2), the dopamine reuptake site and the metabolism of dopamine itself.

Dopamine itself does not cross the blood-brain barrier. Therefore, PET studies of dopamine metabolism have centered on the use of a fluorine-18 labeled derivative of DOPA, 6-[^{18}F]fluoro-DOPA which crosses the blood brain barrier and is metabolized to 6-[^{18}F]fluorodopamine by aromatic amino acid decarboxylase (19). Fluorine-18 labeled derivatives of m-tyrosine, a more stable ligand in vivo is also being examined as a probe for dopamine metabolism (20,21,22). SPECT studies of dopamine metabolism are also of interest and the synthesis of 6-iododopamine has recently been reported (23). Although some animal distribution studies have been performed more biological studies are needed to assess the effect of iodine substitution on this small amino acid.

Dopamine (D_2) receptor activity has also been examined with both PET and SPECT tracers. These basically fall into two general types, the butyrophenones and the benzamides. A large number of compounds have been labeled with carbon-11, fluorine-18 and iodine-123 in search of a radioligand with high selectivity, high uptake into the brain and regional distribution which parallels the distribution of D2 receptors and kinetic properties compatible with kinetic modeling and calculation of relevant receptor parameters (2-4 and references therein). Recently advances have been made in designing and labeling high affinity, highly selective benzamide derivatives with fluorine and radioiodine for PET and SPECT studies of the D2 receptor (24,25,26).

The relationship between dopamine D2 receptors and diseases like schizophrenia is currently being examined with PET in order to confirm the elevation of D2 density found in post-mortem brain samples from schizophrenics (27). Two studies have been carried out using different tracers with one group reporting a significantly elevated Bmax in schizophrenics using [^{11}C]N-methylspiroperidol (28) and another group reports no deviation from normal values using [^{11}C]raclopride (29). The resolution of this apparent discrepancy is now requiring a detailed examination of the methodology used in each of these studies (30). One possible explanation for the results of the measurements with [^{11}C]raclopride is that competion of dopamine for the labeled raclopride may be significant since both dopamine and raclopride have similar Kd's (31,32).

A number of labeled benzazepines have also been developed for PET and SPECT studies of the D1 receptor (2,4 and references therein).

The dopamine reuptake system has also been the subject of radiotracer development. Dopamine reuptake sites appear to be related to neuronal mass and thus radioligands with selectivity for the dopamine reuptake site may be of value in monitoring the progress of neurodegenerative diseases such as Parkinson's disease (33,34). [^{11}C]Nomifensine and [^{18}F]GBR 13119 have been prepared and used to probe the dopamine reuptake system (35,36). [^{11}C]Cocaine, which has been developed to characterize cocaine binding sites in normal human subjects and in chronic cocaine abusers, may also prove useful in longitudinal studies of neurodegenerative disorders (37). A higher affinity ligand for the cocaine receptor, [^{11}C]WIN 35,428 (CFT), is structurally similar to cocaine is also being developed as a PET ligand for the dopamine transporter site (38).

The Muscarinic-Cholinergic System: One of the primary motivations for developing PET and SPECT radiotracers for probing the cholinergic system is its association with the pathophysiology of Alzheimer's disease (39). Beginning with the development of iodine-123 iodoquinudinyl benzilate and the first human study reported in 1984 (8), there has been an active effort in both PET and SPECT research to develop tracers for probing the post-synaptic cholinergic system. A number of PET ligands have been developed. For example [^{11}C]scopolamine has been used to examine the muscarine-cholinergic system but the binding characteristics of this ligand (i.e. rapid transport relative to trapping) have made it difficult to kinetically differentiate the receptor binding compartment (40). Other carbon-11 labeled ligands such as tropanyl benzilate have been developed more recently and show more promise since their extraction does not appear to be flow limited as is the case with labeled scopolamine (41). The anticholinergic drug cogentin has been labeled with carbon-11 and its regional distribution in human brain parallels cholinergic receptors. In addition, its uptake in baboon brain can be reduced by blockade with both cholinergic antagonists and agonists (42). Carbon-11 labeled dexetimide, a potent muscarinic-cholinergic antagonist, and levetimide, its inactive enantiomer have also been prepared for studies of this receptor system (43). Dexetimide and levetimide have also been labeled with iodine-123 and comparative SPECT studies show the appropriate stereoselectivity in human brain validating the use of ^{123}I-dexetimide as A SPECT tracer for the post-synaptic cholinergic system (44).

A new view of the cholinergic system was realized recently with the development of ligands which bind to the cholinergic storage vesicle membrane. Radioiodinated iodobenzvesamicol and [^{11}C](N-methylamino)benzovesamicol show appropriate biological specificity for this application.(45,46). This is a highly promising new development which holds promise for providing a map of the regional distribution of cholinergic neurons. This could provide an important new perspective on neuronal degeneration in Alzheimer's disease.

Other Neurotransmitter Ligands: A number of PET labeled ligands for the opiate system have been developed, among them C-11 labeled carfentanil and diprenorphine (47,48) and F-18 labeled cyclofoxy (49). Labeled carfentanil has been applied to the study of seizure mechanisms where it has recently been demonstrated that mu opiate receptors are increased in temporal lobe epilepsy (50).

Labeled central benzodiazepine receptor ligands are of interest in the study of anxiety and epilepsy. Radiotracers have been developed for studying both central and peripheral benzodiazepine receptors in the brain with PET and SPECT. The selective central benzodiazepine receptor antagonist [^{11}C]RO-15,1788 (51), shows appropriate regional distribution and a dose dependent inhibition of in vivo binding when increasing amounts of unlabeled RO-15,1788 are injected. Iodoflunitrazepam has been developed as a SPECT tracer for the central benzodiazepine receptor (52). Another radioiodinated benzodiazepine, RO 16-0154 has been studied in human brain with SPECT (53,54,55). It shows an in vivo distribution consistent with the distribution of the benzodiazepine receptor and can be displaced in vivo.

A number of ligands which are selective for various post-synaptic serotonin sites have been labeled with carbon-11, fluorine-18 and iodine-123 (2-5 and references therein). In addition, a method for quantitating the rate of serotonin synthesis using carbon-11 labeled alpha-methyl tryptophan has also been recently developed (56).

PET and SPECT tracers for probing the NMDA receptor are under development (57,58,59,60).

Outlook

The prospect of characterizing neurotransmitter properties in normal and disease states holds tremendous promise in terms of understanding neurological and psychiatric illness at the molecular level. This will not only require the development of highly selective tracers but also continual critical examination of the limitations of the in vivo measurments in their ability to provide quantitative information on discrete biochemical transformations. It is safe to say that advances in chemistry and basic research in labeling and characterizing receptor active molecules with PET and SPECT nuclides coupled with advances in imaging instrumentation will continue to play a pivotal role in the development emission tomography as a tool for basic and clinical research.

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