Research and Clinical Potential of Receptor Based Radiopharmaceuticals*

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Receptors are proteins that have specific binding affinity for substances that produce a physiological event in the body. Receptor-binding radiotracers are being used increasingly to study the function of receptors in health and disease. This review summarizes the proceedings of a symposium on research in the development of receptor-binding radiopharmaceuticals. The key phases in this research include: (1) selection of the receptor system and ligand; (2) synthesis of radiolabeled ligand; (3) validation in animal models; and (4) clinical application. Current research involves a variety of biological systems, such as butyrophenone neuroleptics for dopamine receptors and steroidal estrogens for the estrogen receptor. In the future, it is believed that receptor-binding radiopharmaceuticals will be useful, not only to validate receptor systems in vivo, but also to aid in the diagnosis and therapy of human diseases.

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Receptor based radiopharmaceuticals are potential tools for the investigation of the interaction between a radioactive ligand and a receptor in vivo. The use of such tracers is an appealing approach to the study of both the normal function of the receptor and perturbations related to various disease states. This area of research exploits one of the strengths of nuclear medicine relative to other diagnostic modalities—the ability to study physiology and biochemistry in vivo. The tracer technique is particularly suited to the study of receptors since the concentrations of receptors encountered in vivo are generally too low to be assessed by other imaging modalities such as nuclear magnetic resonance (NMR) and computed tomography (CT).

The concept of receptors is usually credited to Langley (1905), with his proposal of "receptive substances" to explain the action of nicotine and curare on muscle contraction (*1*). The concept of receptors was further elaborated by the work of Ehrlich (1906) (*2*) and Clark (1926) (*3*), and since that time the definition of a receptor has been constantly changing. Receptors are currently thought of as high binding affinity proteins with a specific anatomical distribution, that respond to low levels of specific substances with a defined physiological event. Receptors are now accepted as important factors in pharmacology, both for explaining the action of endogenous biochemicals as well as therapeutic pharmaceuticals. Receptors appear to be involved in a bewildering variety of biological systems, ranging from the sense of taste to neuronal transmission.

Changes in the interaction of a ligand with its receptor (e.g., affinity, subtype, receptor concentration) have been increasingly implicated in human disease. For example, postmortem studies using in vitro assays have demonstrated changes in the number of dopamine receptors in patients with various neurological diseases. The use of receptor-binding radiotracers offers a method for in vivo evaluation of basic receptor concepts, diagnostic capabilities, and therapeutic drug regimens. Some possible receptor systems which might be studied are shown in Table 1.

Exploitation of the receptor-ligand interaction is thus an attractive approach to radiopharmaceutical development. In actual practice, progress has been slow. Problems to overcome include the low number of re-

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TABLE 1
Receptor Systems and Diseases for Which Changes in Receptor Biochemistry have been Suggested

Receptor system	Diseases
Dopamine	Parkinson's, dystonia, schizophrenia, Huntington's, Tourette's syndrome
Cholinergic (muscarinic, nicotinic)	Alzheimer's, myasthenia gravis
Opiate	Narcotic addiction, pain syndrome
Serotonin	Sleep disorders
GABA/benzodiazepine	Hemiballismus/hemichorea spasticity
Adrenergic	Depression
Estrogen	Breast cancer
Progesterone	Breast and prostate cancer
Androgen	Prostate cancer
Hepatic cell surface	Liver diseases

ceptors, the complexity of biological systems, and subtlety of changes in receptor biochemistry. In this review, we shall outline the various stages in the development, validation, and application of a potential receptor based radiopharmaceutical.

Selection of receptor system

Not all receptor systems require the use of radiotracers in vivo for their evaluation. In some cases, the clinically valuable information can be obtained by simpler, more convenient approaches (e.g., in vitro assays). If the use of receptor-based radiopharmaceuticals appears warranted, then the receptors must be sufficiently localized, anatomically, to allow their delineation by available instrumentation. Finally, the kinetics of the receptor-ligand interaction must occur in a time frame compatible with isotope half-lives and the constraints of the clinical domain.

Despite these restrictions, quite a few receptor systems have now been studied using receptor-based radiotracers; those which were discussed at this symposium include the dopamine, serotonin, opiate, benzodiazepine, muscarinic acetylcholine, estrogen, hepatic binding protein and norepinephrine receptors.

Selection of ligand

Once the receptor system of interest has been identified, the next step is the choice of the appropriate ligand to label. Most receptors bind a heterogeneous array of compounds with varying degrees of affinity and specificity; fortunately, there is an extensive literature on receptor-ligand interactions, and numerous biochemical techniques are available for evaluation of additional ligands (*4*).

The first criterion for a potential ligand is its binding affinity for the receptor. For many receptor systems, an in vitro preparation of receptor binding sites is available, and the binding affinity of numerous compounds can be easily compared. Competitive binding assays are used to evaluate the ability of a compound to compete with a radiolabeled [hydrogen-3 (^{3}H) or carbon-14 (^{14}C)] reference ligand (e.g., [^{3}H]estradiol for the estrogen receptor). This allows for the screening of a large number of potential ligands without the need to prepare each in radiolabeled form. The data obtained are usually expressed as relative binding affinities (RBA). This approach is of particular value when considering the use of a radiolabeled analog of a high-affinity ligand (i.e., labeling with bromine, iodine, technetium, etc), where radiolabeling may alter the physiochemical properties of the ligand. Katzenellenbogen, using a series of A- and D-ring halogen substituted steroidal estrogens, showed that a smaller substitution of fluorine for hydrogen generally results in more subtle alterations in binding affinities (*5*). Thus, the use of in vitro binding affinity assays provides a valuable first approximation of a radiotracer's potential value, and are especially useful in evaluating multiple potential labeling sites on a candidate ligand. With steroidal estrogens, for example, substitution in the 16-position (D-ring) is clearly better than modifications in the 2- or 4-position of the A-ring. Similarly, iodination of quiniclidyl benzilate in the 4-position was identified by Eckelman as the best of the several possible iodinated QNB derivatives (*6,7*). Finally, Wagner and associates used in vitro assays to demonstrate the potential of a methylated derivative of spiroperidol as a ligand for studying the dopamine receptor (*8*).

In nuclear medicine, successful imaging requires adequate contrast between the anatomical target and surrounding, background tissues. With receptor based radiopharmaceuticals, there are several additional sources of background which can be present within the target tissue itself. These include the following; (1) high affinity binding of the ligand to other receptors present in the tissue, (2) high affinity nonreceptor binding, and (3) low affinity, nonsaturable nonspecific binding. (Two other sources of background, unbound ligand and labeled ligand metabolites, will be discussed later).

The first source of background, cross-reactivity with nontarget receptors, is exemplified by the binding of butyrophenone neuroleptics (high affinity dopamine receptor ligands) to serotonin receptors. This is not a general problem; for example, it is not encountered with radiolabeled estrogens, but might be a source of background with androgens and progestins, where considerable cross-binding has been observed.

The second source of background is high affinity binding to nonreceptor sites, which is exemplified by the binding of compounds to specific plasma proteins. As an example, Katzenellenbogen has described the binding of steroidal estrogens to sex steroid binding globulin (*4*).

This type of binding can in some cases be diminished by chemical modification of the ligand structure; for the steroidal estrogens, substitution at the 11β-position effectively abolishes binding to specific plasma proteins.

The third contribution to background, and perhaps the most important, is nonsaturable nonspecific binding. This is attributable to the binding of lipophilic compounds to hydrophobic sites which are ubiquitous in proteins, membranes, and other biological structures. Katzenellenbogen discussed one approach which allows a priori estimation of non-specific binding, based on the compound's lipophilicity. Using the pharmacological approach of Hansch and coworkers (*9*), nonspecific binding is approximated by the binding of a compound to human serum albumin. With this method, the octanol:water partition coefficients (log P) of a series of compounds are either measured or calculated, and the log P values then used to calculate the expected affinities for serum albumin. In general this method correlates well with actual measurements of binding to serum albumin determined by equilibrium dialysis.

Since the nonspecific binding of a potential radiotracer is often the largest component of the background, in practical terms the combination of relative binding affinity and some measure of nonspecific binding might offer a more useful indication of potential value than just relative binding affinity. Katzenellenbogen and colleagues have defined a term called the binding selectivity index (BSI), which is the ratio of relative binding affinity (RBA) to non-specific binding to albumin (NSB) (*4*). In Fig. 1 the RBA, NSB, and BSI values for a series of halogenated steroidal and nonsteroidal estrogens are shown. This approach was used to identify certain ligands (16-halo-estradiols, side-chain fluorinated hexestrols) as good candidates for labeling with bromine-77 (^{77}Br) or fluorine-18 (^{18}F). The value of the BSI approach, at least for the radiolabeled estrogens, is exemplified by the observation that the target to nontarget ratios obtained in vivo correlated much better with the BSI values than the RBA values.

This approach is not necessarily applicable to all situations. The very determination of RBA values by in vitro binding assays may have a considerable component that can be attributed to differences in lipophilicity. In at least one case, that of *N*-methylspiperone, the slight increase in log P caused by the chemical modification (the methylation of the amide nitrogen in spiroperidol) may be an improvement, because more of this ligand crosses the blood brain barrier and is potentially available for interaction with the receptors.

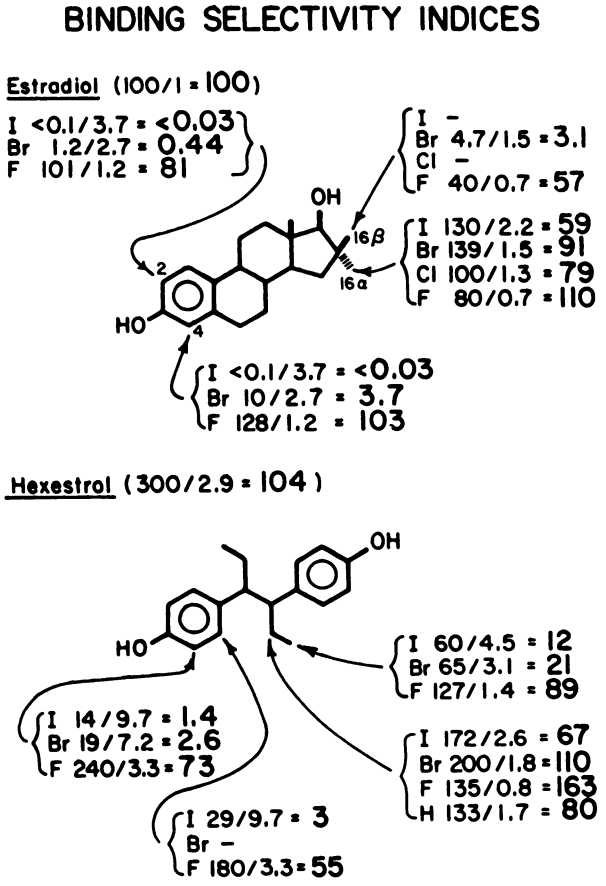

FIGURE 1
Binding selectivity indices (BSI) for halogenated steroidal and nonsteroidal estrogens. Values shown are in form RBA/NSB = BSI, where RBA = relative binding affinity (normalized to estradiol = 100) and NSB = nonspecific binding (normalized to estradiol = 1.0)

Synthesis of labeled ligand

Using the aforementioned in vitro techniques, it is possible to identify ligands (perhaps several) which are good candidates for radiolabeling. The next step is to prepare the radiolabeled ligand in a practical yield, with purity (chemical and radiochemical) and specific activity suitable for in vivo evaluation.

The choice of radionuclide depends on the following factors: availability of radionuclide, imaging device to be employed (PET or SPECT), time course of the biochemical process, available radiochemical syntheses, and specific activity required.

Appropriate isotopes for receptor studies with positron emission tomography (PET) include ^{18}F, ^{75}Br, and ^{76}Br. If single-photon studies are planned, ^{77}Br, iodine-123 (^{123}I), and technetium-99m (^{99m}Tc) are the isotopes most often utilized. If the time course of the study is short (<90 min), carbon-11 (^{11}C), with a 20-min half-life, may be adequate. Wolf and coworkers have demonstrated that ^{11}C has the advantage of allowing repetitive studies in a single subject on the same day, by allowing for decay of the isotope between studies (*10*). On the other hand, if the receptor kinetics demand a longer time span of investigation, then a longer-lived radionuclide will be required to maintain good counting statistics throughout the desired imaging period.

At present, both ^{11}C and ^{18}F can be prepared in quantities and chemical forms suitable for radiopharmaceutical synthesis. Carbon-11 can be routinely prepared on small medical cyclotrons capable of producing 15-17 MeV protons. Two ^{11}C-labeled precursors, ^{11}C-methyl iodide and hydrogen ^{11}C-cyanide, are easily and rapidly obtained by on-line chemical syntheses. The labeling of compounds with ^{11}C is often fairly straightforward and well-precendented in syntheses with carbon-12, carbon-13 or carbon-14. Quite a large number of potential radioligands have been labeled with ^{11}C. As examples, Wolf and coworkers have described the synthesis of ^{11}C-labeled spiroperidol (using $H^{11}CN$) (*11*), and Comar described the syntheses of Ro151788 and flunitrazepam (ligands for benzodiazepine receptor) (*12*) and ^{11}C-methylquiniclidyl benzilate (*13*), both prepared using $^{11}CH_3I$. The use of ^{11}C has one distinct advantage over almost all other radionuclides: incorporation into an organic compound is an isotopic substitution, and can be made without changing the chemical or physiochemical properties. One of the difficulties with ^{11}C, however, is the relatively low specific activities obtained.

For many years, ^{18}F has been produced using neon gas cyclotron targets, and obtained as either ^{18}F-F_2 or $H^{18}F$. Fluorine-18 labeled F_2, however, is of low specific activity (~12 Ci/mmol), and syntheses have a maximum radiochemical yield of 50%. Neon gas targets for $H^{18}F$ production have been reported by many workers, but the reliability of these targets for producing the needed quantities of ^{18}F has, at times, been disappointing. Recently, high specific activity ^{18}F-fluoride ion has been produced by way of the proton irradiation of an oxygen-18 enriched water target. This method has finally made available on a wide-spread basis a suitable source of ^{18}F for radiotracer synthesis. Not surprisingly, most of the current preparations of ^{18}F labeled high specific activity radioligands have utilized this source of ^{18}F. Fluoride ion displacements of alkyl trifluoromethanesulfonates, bromides, and cyclic sulfates have been used to prepare ^{18}F-labeled alkyl fluorides, such as [^{18}F]-16α-fluoroestradiol. For aromatic fluorinations, the Balz-Shiemann and triazene decomposition reactions (which have the drawbacks of either low specific activity or very low radiochemical yields) have been superceded by the use of aromatic nucleophilic substitution, a reaction which produces high specific activity [^{18}F]aryl fluorides in good to excellent yields. At present, this is the method of choice for preparation of the butyrophenone neuroleptics [^{18}F]spiroperidol and [^{18}F]*N*-methylspiroperidol.

Although (fortuitously) several of the neuroleptic drugs being utilized contain fluorine, in most cases fluorine should, like the other halogens, be considered a foreign label. Also, fluorine chemistry is often much more difficult than labeling with ^{11}C, or one of the heavier halogens. However, the fairly long half-life (110 min) of ^{18}F does allow a longer time for both synthesis and purification of the radioligand.

Perhaps the most significant advantages of ^{77}Br and ^{123}I are their suitability for use with single photon instrumentation, and the longer half-lives which make them suitable for use at institutions distant from their sites of production. These halogens must in almost every case be considered as foreign labels, but fortunately there is considerable information in the literature on the use of iodine-125-labeled ligands both in vitro and in vivo. Halogenations with the heavier halides are usually simpler and often more successful than the corresponding fluorinations. For example, triazene decompositions have found little utility in ^{18}F-fluorinations of all but the simplest compounds, but have proven very useful in brominations and iodinations, as in the preparation of 4-iodo-quiniclidyl benzilate. It is important to remember that iodination and bromination may significantly alter the physiochemical properties of a ligand (lipophilicity, size), making the application of appropriate in vitro assays (discussed earlier) a vital part of the radiopharmaceutical development.

Specific activity is a general consideration in radiopharmaceutical design, usually due to questions of drug toxicity. The low in vivo concentration of receptors, coupled with the sometimes acute pharmacological effect of extremely low doses of receptor-binding drugs, dictates a need for high specific activity in the synthesis of receptor based radiopharmaceuticals. What will be the required specific activity, however, depends on the system to be studied. An estimation of the required specific activity has three components: (1) the concentration of receptors in the target tissue (usually pmole/cc); (2) the amount of radioactivity per unit volume needed to be successfully imaged by available instrumentation; and (3) the fractional occupation of the receptor which can be tolerated without a pharmacological response.

Two examples of this type of calculation are the following:

1. For estrogen-dependent tumors, in vitro assays have shown estrogen receptor concentrations around 1 pmol/cc. If 0.1 μCi/cc is needed for imaging with a particular PET or SPECT machine, and the assumption of 10% occupation of receptors is made, then a minimum specific activity of 1,000 Ci/mmol would be necessary.

2. For a system which has a higher concentration of receptors (15 pmol/cc) and using a more sensitive imaging device (requiring only 0.01 microcurie/cc), then even with the restriction of only 2% receptor saturation (very low receptor occupancy may be required for neuroreceptors), the necessary specific activity is only 33 Ci/mmol.

All of the radionuclides discussed in this review can be obtained in specific activities greater than 1,000 Ci/

mmol. The most difficult radionuclide from a specific activity standpoint is ^{11}C, where the ubiquitous nature of carbon-12 in gases, reagents, apparatus, etc., causes considerable dilution of the radionuclide. One of the distinct advantages of the radiohalogens is the ability to achieve much higher specific activities in both synthetic precursors and final radiolabeled ligands than is possible with ^{11}C.

With receptor-binding radiotracers, it is important to distinguish the difference between the specific activity of a radioligand determined by a physical measurement (e.g., uv absorbance) and the "effective" specific activity determined by an in vitro receptor binding assay. In all cases when both have been determined, the effective specific activity has been lower (sometimes considerably so) than the chemical specific activity. This has been attributed to the presence of unlabeled, chemically similar species which are not separated in the purification process, but which have an affinity for the receptor binding site. For example, the effective specific activity of [^{123}I]-4-iodoquiniclidyl benzilate was about one-third of that determined by the uv detector. This was the result of a minute, but finite tailing of unlabeled QNB underneath the later-eluting IQNB peak during high performance liquid chromatography purification.

An additional consideration in the choice of a labeled radioligand is how it may be metabolized in vivo. Metabolic stability can be a factor both in the choice of which isotope to utilize and where in the structure of a ligand to place the label. Although certain trends have been observed, it is difficult to always predict the metabolic stability of a potential radioligand; in this respect, the pharmacological literature is indispensable. Consider as an example a common method of ^{11}C labeling, the alkylation of a nitrogen (amine, amide) with $^{11}CH_3I$. In some cases the product is metabolically unstable in vivo, since the ligand is rapidly de-methylated. In other cases, such as the compound (^{11}C-*N*-methyl)Ro151788 described by Comar, the radioligand does not undergo appreciable in vivo demethylation (only 2–3% in 1 hr). A more complex example is *N*-methylspiroperidol, which has been labeled with both ^{18}F and ^{11}C by Wolf and co-workers (unpublished data). These two radioligands give different sets of radiolabeled metabolites, with more metabolites observed for the ^{11}C-labeled species. The production of labeled metabolites in vivo has important ramifications in the imaging process, since these products have the potential of contributing to the background in both target and non-target tissues. Preliminary studies have shown that labeled metabolites of ^{18}F-labeled butyrophenone neuroleptics do not accumulate in target (striatum) or nontarget (cerebellum) tissues in the brain; presumably the more polar metabolites are excluded by the blood-brain barrier. This may not be the case with other radioligands and tissues; for example, with [^{18}F]16-α-fluoroestradiol, the labeled metabolites are not found (<5%) in the target tissue, the uterus, but are present in large percentages (90%) in nontarget tissue such as muscle (1 hr postinjection).

Validation in animal model

Once a potential receptor-binding radiopharmaceutical has been designed, synthesized and evaluated in vitro, the next step is the demonstration of in vivo receptor-mediated uptake in an appropriate animal model. It is necessary not only to show radiotracer uptake into receptor-rich anatomical structures, but also to demonstrate that this uptake is both saturable and displaceable by a high affinity antagonist.

The selective uptake of a receptor-targeted radiotracer into receptor-rich tissue is usually easy to demonstrate. For example, with radiolabeled (^{11}C, ^{18}F, ^{75}Br, ^{76}Br) dopamine receptor antagonists (*8,10,14–16*), the enhanced uptake into the striatum, relative to other regions of the brain, is observed by either ex vivo (removal of tissues) or in vivo (imaging) techniques. Similarly, uptake of radiolabeled (^{77}Br, ^{18}F) estrogens into a specific target organ (uterus) has been achieved (*5,17*), and ^{99m}Tc-galactosylneoglycoalbumin (NGA) shows uptake and retention of label only in the targeted organ, the liver (*18,19*). Anatomic specificity has been demonstrated for ligands for the benzodiazepine, opiate, and muscarinic acetylcholine receptors.

The next step is to demonstrate that this anatomic specificity is indeed a result of a receptor-mediated process. This is accomplished by showing that the uptake of radioactivity is dose-dependent: with the extremely low levels of receptors present, it should be possible to saturate the available binding sites. These experiments involve either determination of a dose-response curve (response being radioactivity uptake), or, in a simpler experiment, the blocking of radiotracer uptake by either pre- or coinjection of sufficient unlabeled ligand to saturate the receptors present. Again, this type of experiment has been reported for virtually all of the receptor-binding radiopharmaceuticals.

A similar, but slightly different validation experiment, is the displacement of bound radioligand in vivo by an injection of a competing, high affinity antagonist. In this case, the receptor-bound radioactivity is displaced, with rebinding to the receptor blocked by the presence of higher concentrations of the competing ligand. For example, the binding of Ro151788 to the benzodiazepine receptor can be displaced in the baboon by injection of flunitrazepam as the competing ligand.

In special cases it may be possible to exploit a stereospecificity in the binding of a radioligand to the receptor in vivo. As reported by Eckelman (*6*), [^{123}I]-4-iodoquiniclidyl benzilate can be prepared in two stereoisomeric forms, termed R and S; only the R form shows high affinity binding to muscarinic receptors. Both of these stereoisomers have now been studied in vivo. Ini-

tially, the two stereoisomers are ditributed in a similar manner, reflecting blood flow and lipophilicity (factors independent of receptor stereospecificity); as time progresses, only the R-isomer, with the much higher RBA value, accumulates in the receptor rich regions. This type of experiment permits measurement of both receptor-based uptake of radioligand and nonspecific binding in the target tissue. Unfortunately, stereoisomers of radioligands with radically different binding affinity values are not always available, or the synthesis of such would be difficult and time-consuming. In addition, the use of two radiotracers would increase the complexity of clinical studies.

Finally, receptor systems may be species specific, as demonstrated by Stadalnik. Technetium-99m NGA binds in receptor specific fashion to hepatic binding protein which is found in mammalian liver. However, no specific uptake is observed in an avian system (chicken).

Clinical applications

With regard to the clinical application of receptor-based radiopharmaceuticals, the complexity of the study will be dependent on the clinical or physiological data which is desired by the investigator. In some cases, the simple demonstration of relative changes in uptake of the radioligand into receptor-rich anatomical structures may be sufficient. Conversely, if detailed, quantitative information about the receptor concentrations and kinetics of radiotracer uptake is desired, then a more complex study will be required, and the data obtained analyzed by a mathematical model.

The first approach, that of demonstrating anatomically specific uptake of a receptor-based radiopharmaceutical in humans, has been accomplished using a variety of ligands, radionuclides, and imaging devices. In Fig. 2 is shown, as an example, a SPECT study showing the selective localization of [^{123}I]-4-iodo QNB in the caudate nucleus of a human volunteer. Similarly, the localization of [^{11}C]N-methylspiperone in the dopamine receptor rich striatum has been demonstrated in a large series of volunteers and patients by Wagner and colleagues (*8*,*20*); uptake of [^{77}Br]estradiol into estrogen-receptor rich breast tumors shown by Katzenellenbogen and co-workers (*17*); in vivo binding of [^{11}C]-Ro17588 to benzodiazepine receptors shown by Comar and coworkers; and specific liver uptake of ^{99m}Tc-NGA in human volunteers and patients shown by Stadalnik (*18*,*19*). These are not by any means the only examples of human studies now being undertaken with receptor-based radiopharmaceuticals.

These studies in humans are the first demonstration of the great potential of receptor based radiopharmaceuticals. For the first time, data showing the regional localization and binding kinetics of receptors in vivo have been obtained and the results agree very well with those

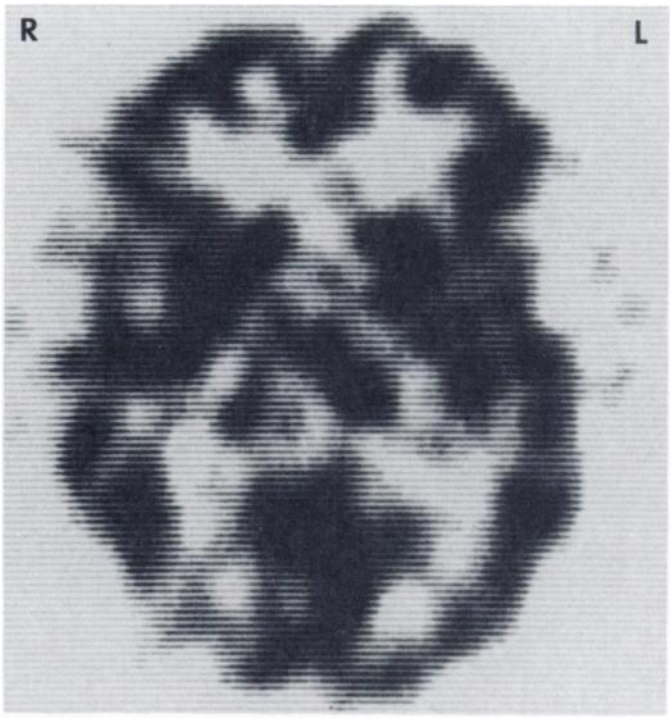

FIGURE 2
Single photon emission tomographic image obtained after injection of 4-[^{123}I]iodoquiniclidyl benzilate in human volunteer (Reprinted by permission, Ref. 7)

previously attainable only from biopsy or autopsy specimens using in vitro analyses (*15*,*20*). Such information is not accessible by other medical techniques. For example, Wagner and associates (*20*) have reported, using [^{11}C]N-methylspiperone, an age-dependent decrease in the uptake of the radiotracer into the striatum: this finding is consistent with data from autopsy studies, which have shown a decrease in the number of dopamine receptors with increasing age. In preliminary studies, the Johns Hopkins group has shown decreased uptake of [^{11}C]N-methylspiperone in the caudate nucleus of patients with Huntingdon's disease. As an example of a completely different receptor system, Stadalnik and coworkers have demonstrated clinical potential of Tc 99m-NGA liver imaging in studying cirrhosis, hepatoma, hepatic metastases, liver transplant, and hepatitis (*18*,*19*). These results are but examples of the potential of this approach in nuclear medicine.

Disease-associated changes of receptor characteristics may be very subtle, and a careful and thorough analysis of in vivo data may improve the sensitivity of these techniques. The uptake of radioactivity into a receptor-rich tissue depends not only on receptor concentration, but also on ligand delivery (blood flow), transfer from the vascular compartment into the tissue (permeability), availability of the ligand in the tissue for binding with the desired receptor, and the kinetics of the ligand-receptor interaction. For example, blocking ex-

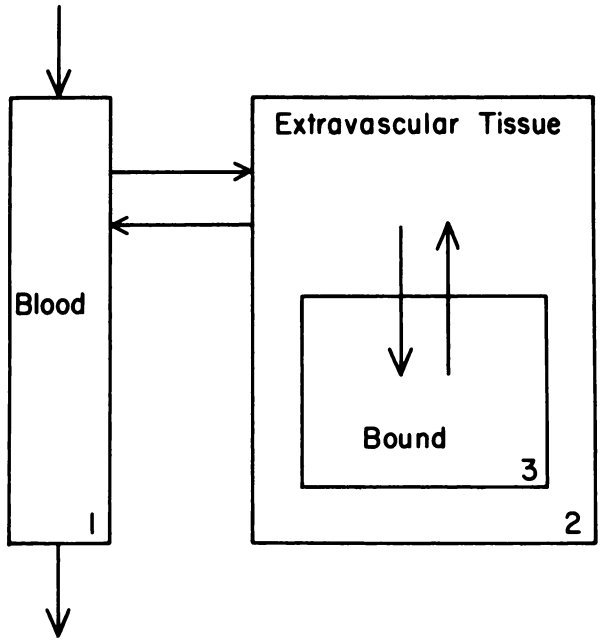

FIGURE 3
Three-compartment model used in analyses of data obtained after injection of receptor-based radiopharmaceuticals. Compartment 1 represents vascular space, compartment 2 the extravascular space, and compartment 3 represents receptor-bound radioactivity

periments using (+)butaclamol have shown that not all of the uptake of radiolabeled neuroleptics into the striatum represents receptor bound radioactivity.

Unraveling the various components of tracer uptake requires the use of mathematical models. The model most often used is the three compartment model, which is depicted in Fig. 3. The radioactivity in a region of interest can be assigned to one of three compartments: vascular activity, radioactivity in the tissue but not bound to receptor, and receptor-bound radioligand. Mathematical equations can then be derived to describe the movement of radioactivity between the various compartments. In such an approach, there are numerous variables to be determined: blood flow, blood volume, permeability, ligand binding to nonreceptor sites in blood and tissues, and receptor kinetic values (association and dissociation rates).

One approach, taken by Raichle and coworkers (*15*), has been to solve for these variables making minimal assumptions. This method requires independent measurements of some variables (blood flow and blood volume), and obtaining kinetic data over a 2-3 hr time period. These investigators use extensive computer calculations (parameter estimation) to solve for a value they term the binding potential, BP, which is defined as the total number of binding sites or receptors multiplied by their affinity for the ligand ($B_{max} \cdot K_D^{-1}$). This term thus represents the capacity of a tracer to bind a specific receptor ligand.

There are several aspects to this rigorous approach, particularly the need for independent measurement of several variables, accurate determination of blood activity levels (correcting for metabolites), and lengthy acquisition of kinetic data (2 to 3 hr), which may make it inconvenient for clinical studies. Simpler solutions for the three compartment model have thus been sought, and one such approach has been proposed by Wagner and coworkers (*8*). Their simplification of the model requires several assumptions: the dissociation rate from the receptor is zero, the rate at which ligand exits the tissue is much faster than the rate of association to ligand, and, finally, the plasma radioligand concentration becomes constant after a period of time. Using these assumptions, the Johns Hopkins group has suggested that the slope of the line obtained by plotting the striatum-to-cerebellum ratio versus time is a reflection of the fractional rate constant (termed k_3 by these researchers) governing the binding of the ligand to the receptor. Such a simplification is very appealing and may, in some instances, be a useful indicator of properties of the receptor binding process. It should be noted that k_3 represents, in classical receptor pharmacology, the product of the number of receptors, the forward rate constant, and the fraction of ligand available for binding.

In a recent set of experiments, described by Raichle (Raichle et al., unpublished data), the simplified and rigorous solutions of the three-compartment model were applied to the same in vivo data obtained following injection of [^{18}F]spiroperidol in baboons; the k_3 value was then calculated using each of these approaches. The results obtained using the simple and rigorous model solutions did not agree. The divergence of the results was attributed by Raichle to a changing plasma concentration of radioligand and, possibly, a small but finite dissociation rate for the ligand from the receptors.

At this stage in the development of receptor-binding radiopharmaceuticals, an understanding of the appropriate use of mathematical models is of crucial importance. It is clear, however, that such models will yield previously unattainable information about many of the characteristics of receptors in vivo.

Future potential

The research and clinical applications of receptor-based radiopharmaceuticals are only just beginning. The potential future uses of these radiotracers will include three types of studies. First, they will offer scientists the ability to validate basic biochemical constructs of receptor systems through study of these receptors in their natural, in vivo environment. Second, it may prove possible to aid in the diagnosis of human diseases, ranging from cancer to neurological disorders, which appear to be related to changes in specific receptor systems. Fi-

nally, studies with receptor-based radiopharmaceuticals might be useful in assessing the effectiveness of therapeutic regimens for such diseases. The ability to study receptors in vivo and to explore their characteristics and relationship to human diseases is indeed an exciting prospect.

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