Editorial Comment

PET Receptors

Counting Receptors Using Positron Emission Tomography

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Receptors control many aspects of cardiac metabolism and function, and alterations in the number of receptors may play an important role in the control of cardiac function and the pathogenesis of disease. The availability of radiolabeled ligands in the 1970s made it possible to directly measure receptor number and affinity. These methods are restricted to cardiac tissue that can be studied in vitro either from animals or human tissue obtained at autopsy or biopsy. Thus, clinical investigations are limited to animal models of disease or settings in which cardiac biopsy or surgery is performed. Despite this limitation, these types of studies have led to important insights into receptor regulation in the heart.1–4

Another approach has been to study circulating blood cells (leukocytes or platelets) as models for inaccessible organs. While easy to perform, these studies can be difficult to interpret, as receptors on blood cells can be regulated differently than those in the heart.5 Moreover, white blood cells are heterogeneous, and changes in receptor expression can be caused either by changes in intracellular receptor expression or a change in the population of cells that circulate in the blood.6 Furthermore, blood cells do not contain most of the receptors of interest in the heart; although platelets have α2-adrenergic receptors and leukocytes have β2-adrenergic and histamine receptors, muscarinic cholinergic, α1-adrenergic, β1-adrenergic, and dopamine receptors do not appear to be present in large numbers on blood cells.

Positron emission tomographic (PET) scanning offers the possibility of noninvasive measurements of cardiac receptors in individual patients (for review, see References 7 and 8). While the details are complex, the principles of PET scanning can be easily understood. Positron-emitting isotopes are generated in a cyclotron by bombarding the target with protons. The proton displaces a neutron in the nucleus. The nucleus of the resulting atom has too many protons (compared with neutrons) and is thus unstable. The proton turns into a neutron, emitting the positive charge and excess energy as a positron. A positron has all the properties of an electron except that it is positively charged; it is also called an antielectron. The positron loses its energy by ionizing or exciting various molecules it bumps into and then combines with an electron. The electron and positron interact briefly, and then annihilate each other. Their energy converts to two photons emitted in opposite directions. The PET detectors are designed to measure the simultaneous emission of these two photons. The positron can travel a short distance before annihilation; consequently, the spatial resolution of PET scanning is limited to about 1 cm.

Preparation of appropriately labeled probes for PET scanning is a challenge because the half-life of positron emitting isotopes is short. For example, carbon-11 has a half-life of only 20 minutes. Thus, the isotope must be generated on site, and the ligand synthesized and purified quickly. Despite these obstacles, positron-labeled ligands have been synthesized for binding to several types of receptors, including muscarinic cholinergic, β-adrenergic, benzodiazepine, and dopamine receptors.9 Other types of positron-emitting probes have been used to measure blood flow, glucose, and fatty acid metabolism in the heart.10,11 Most work with PET has been in the brain.

PET scanning can yield information about both the distribution and number of receptors. However, obtaining this information is quite difficult. The methodology used in conventional radioligand binding studies is not appropriate, as these protocols all involve replicate samples and a method for separating bound from free ligands. With PET scanning, all that can be measured is the amount of ligand present in a region of interest at various times. Some of this ligand is in the blood, some in the extracellular fluid, some nonspecifically bound to cells, and some specifically bound to receptors. The challenge in PET scanning is to extract receptor number, distribution, and affinity from such data. This procedure requires constructing a mathematical model, designing a suf-

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ciently complicated experimental protocol, and conducting a sophisticated data analysis.

The simplest protocols are equilibrium studies using a single concentration of ligand. However, such protocols cannot determine both receptor number and affinity, as identical results are obtained when ligand binds to a large number of low-affinity receptors or a small number of high-affinity receptors. However, the ratio of $B_{\text{max}}$ to $K_d$ can be determined (assuming that receptor occupancy is low), and this ratio is called the "binding potential."\(^{12}\)

A kinetic, nonequilibrium approach must be used to untangle receptor number and affinity. In this issue of Circulation, Delforge et al develop such a method for measuring the number and affinity of muscarinic cholinergic receptors in dog heart.\(^{13}\) They assume a model in which the ligand reversibly diffuses between blood and an extravascular extracellular compartment from which it can bind either to receptor (reversible) or nonspecific (pseudoreversible) sites. Despite its apparent simplicity, this model has six parameters: diffusion rate constants in and out of the extravascular extracellular compartment, association and dissociation rate constants for receptor binding (whose ratio is receptor affinity), fraction of ligand bound nonspecifically, and receptor number. The authors have devised a clever experimental protocol that provides sufficient data to determine values for all six parameters from a single experiment.

First, a small dose of the ligand is injected, and the accumulation in the heart is followed. (This gives information about the input kinetics—diffusion into the extravascular extracellular compartment and binding to the receptors.) Next, unlabeled ligand is injected, and the rate at which labeled ligand leaves the heart is followed. (This gives information about the dissociation rate of the ligand from the receptor and diffusion out of the compartment.) The authors have previously shown that data obtained from this truncated protocol do not unambiguously define values for all six parameters.\(^{14}\) Therefore, they have extended the protocol as follows. Next, labeled and unlabeled ligand are coinjected. As the receptors are occupied by unlabeled ligand, this part of the protocol gives information about the diffusion of ligand into the interstitial space. Finally, a much larger concentration of unlabeled ligand is injected to determine the extent of nonspecific binding. Although it is helpful to consider each part of the experiment as yielding information about different parameters in the model, all the data were fit simultaneously to determine all the parameters using a nonlinear regression computer program.

By means of a sophisticated data analysis procedure, the authors were able to analyze the data and obtain a unique solution with reasonable values for all the parameters. The predictions of the model closely match the experimental data (compare Figures 3 and 4).

Several criteria can be used to confirm that the values determined by the computerized analysis are reasonable. First, the standard error of each parameter is reasonably low. Second, similar results were obtained when either high or low amounts of the radiolabeled ligand were used (the injected dose spanned a sixfold range). Third, the values of the association and dissociation rate constants for ligand binding to receptors are quite close to values determined in vitro, and the receptor affinity is therefore also quite close to values determined in vitro. The results for $B_{\text{max}}$, the receptor number, were about fourfold higher than in vitro results; this may be due to receptors lost or destroyed when the membranes are prepared.

When the labeled probe is injected, it soon concentrates in the heart and stays there for many minutes. The obvious conclusion is that the probe binds tightly to the receptor. However, the authors show that this conclusion is wrong. Instead, after the ligand dissociates from one receptor, it rapidly rebinds to another nearby receptor. Thus, although the half-life by which a ligand dissociates from a particular receptor is about 3 minutes, the half-life by which the ligand leaves the heart is many times longer. This observation may necessitate some rethinking of early PET scanning data. This concept may also be useful when thinking about the pharmacodynamics of unlabeled drugs. The duration of a receptor antagonist in an organ, and thus its physiological effects, may be far longer than its half-life in the plasma.

The authors also note that the amount of receptor binding in various parts of the heart is largely limited by blood flow. Thus, a PET image (before equilibrium is reached) would not be primarily a map of receptor location; instead, it would primarily be a map of blood flow in the heart. This observation may also necessitate some rethinking of early PET studies.

The full protocol takes more than two hours and involves four injections. However, once the various parameters are identified using the full protocol, the authors suggest that it will be possible to constrain the model so as to use simplified protocol (omitting the last two injections). Such a shortened protocol should be quite feasible for use in clinical studies. Since appropriately labeled probes can be synthesized for binding to many types of receptors and PET equipment is available in many centers, it should be possible to adapt the experimental protocol and mathematical model presented by Delforge et al to other types of receptors. This adaptation should provide a means to noninvasively measure the density of various types of receptors in the hearts (or other organs) of patients.

The advantages of PET scanning of receptors are clear. Once the methodology is validated, it should be possible to directly measure receptor number in human hearts (or other organs) in vivo without the need for biopsy. The disadvantages of measuring receptors with PET are also clear: 1) PET scanning requires sophisticated equipment (including a cyclotron) and a large team of experts and thus is
extremely expensive. 2) PET scanning exposes the patient to a small amount of radiation. 3) Fewer manipulations are possible than with in vitro studies. Thus, many types of experiments that are simple with in vitro methods are unfeasible with PET studies (e.g., competing for ligand binding with a variety of concentrations of an unlabeled drug to determine receptor subtypes or with an unlabeled agonist to measure agonist affinity). 4) The endogenous agonist (in this case acetylcholine) is present during the studies and may interfere with ligand binding to an unknown and variable degree. 5) Data analysis is cumbersome and requires complicated models, making it more difficult to design new types of experiments. 6) The expense and complexity of PET essentially prohibit an investigator from performing speculative experiments based on a hunch. 7) While PET can give information about the number of receptors in a region of the heart, it cannot discriminate between myocytes, fibroblasts, endothelial cells, and blood cells.

Will receptor quantitation with PET scanning replace conventional radioligand binding assays? PET methodology is powerful, and its availability will be particularly useful in clinical studies of diseases for which no good animal model exists. However, the expense and complexity of the technique are substantial, and conventional in vitro radioligand techniques are likely to remain the mainstay of receptor research in the foreseeable future.

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PET receptors. Counting receptors using positron emission tomography.
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Circulation. 1990;82:1536-1538
doi: 10.1161/01.CIR.82.4.1536

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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