Functional Imaging of Physiological Processes by Positron Emission Tomography

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Tracer technology makes it possible to observe physiological and biochemical processes in the living organism in a dynamic mode. Positron emission tomography adds the use of chemically unchanged biomolecules and of quantification. This opens up fascinating possibilities for both fundamental research and routine diagnostic applications.

The ability to observe physiological and biochemical processes in the living organism in a dynamic mode opens up fascinating possibilities for both fundamental research and routine diagnostic applications. This can be done by tracer technology: an atom in the molecule to be investigated in vivo is replaced by a an isotope, which can be detected from outside the body (9). Ideally the tracer is chemically identical with the original atom, thus ensuring that the biochemical process to be studied is not changed.

Biologically interesting molecules mainly consist of carbon (C), hydrogen (H), nitrogen (N), and/or oxygen (O). It is possible to use stable isotopes of these elements and to look at them with magnetic resonance imaging and nuclear magnetic resonance spectroscopy at picomolar concentrations ($10^{15}$ molecules/l) or higher. Usually the molecules of interest are at lower concentrations, and the only way to observe them is by positron emission tomography (PET). A well-defined stable C, N, or O atom in a molecule is replaced by $^{11}$C, $^{13}$N, or $^{15}$O, respectively. These radioisotopes are special because of their decay by positron emission and their short half-life ($^{11}$C, 20 min; $^{13}$N, 10 min; $^{15}$O, 2 min). Therefore, the production of the radionuclides (for which a cyclotron is needed) and their introduction into the desired molecules has to be performed at the place of application [usually a hospital; note that cyclotrons generate a large amount of dangerous ionizing radiation and have to be operated behind heavy shielding (9)].

In a cyclotron, ions of hydrogen, deuterium, or helium are accelerated in a vacuum chamber by an electric field, while their path of movement is controlled by a magnetic field. As the particles reach the energy required for the chosen nuclear reaction, the particles are extracted from the vacuum chamber and deflected to hit a target, which contains the material to be converted into the desired radionuclide. Thus $^{11}$C can be produced by shooting a proton (H$^+$) into the nucleus of a $^{14}$N atom. The excess energy in the nucleus is released by the expulsion of a high-energy alpha particle (a “He$^{2+}$ ion), leaving behind the $^{11}$C atom (nuclear reaction: $^{14}$N (p,$\alpha$) $^{11}$C).

To enable the study of longer biological processes and transportation of the labeled products to neighboring institutions, $^{18}$F (half-life ~ 2 h) is also used in addition to the very-short-lived natural elements. In many molecules, it is possible to replace a hydrogen atom or a hydroxyl group with fluorine without changing the pertinent biological properties.

**PET: some theoretical background**

Positron emission enables tomographic imaging (PET), the dynamic registration of the radioactivity distribution in the body (10, 12). A positron is a particle with the same mass as the electron but with a positive electric charge. After being emitted from the atomic nucleus, it has to lose its kinetic energy, and then it combines with an electron. Both particles are annihilated, emitting two 511-keV gamma radiations in opposite ($180^\circ$) directions. If in two detectors placed opposite each other a 511-keV gamma is registered at the same time (coincident), an annihilation must have occurred on the line connecting the detectors (Fig. 1). If many detectors are arranged in rings, forming a cylinder, the events can be recognized in three dimensions. From these data, the spatial distribution of the radioactivity in the body can be reconstructed using appropriate computer algorithms.

Radiation is absorbed by tissue according to its mass. Taking this into account, the measured radioactivity can be quantified. The absorption can be determined by a special measurement in the PET scanner or by computer tomography (CT). Now these measurements can be taken in a new, combined PET-CT apparatus, which as an added bonus enables improved fusion of morphology (CT) and biochemical and physiological function (PET).

Dynamic data can be collected in specific biological (sub)structures (regions of interest) by recording the radioactivity over time. To describe the supply (input function) of a compound studied, the concentration in arterial blood is measured. Comparison of these regional time activity curves with theoretical models allows the calculation of biological functions like oxygen consumption or sugar metabolism, depending on the applied molecule. This procedure is known as pharmacokinetic modeling (2) and requires detailed knowledge about physiology and metabolism of the applied tracer molecule. Because only the absolute radioactivity in the target tissue is measured, it must be determined if the radioactivity is associated with the intact applied substance or with its metabolites. This is done by rapid chemical analysis (high-performance liquid chromatography) of blood and (if possible).
tissue samples. Quantitative evaluation becomes mostly impossible if metabolites are also taken up by the target organ or if metabolism in the target organ occurs.

Because of the limited precision of the PET measurements, only a small number of parameters can be calculated from the data. Thus usually a two- or three-compartment model is used, which is mostly a considerable simplification of the actual biological process.

Production of molecules used with PET

The time available for the labeling of PET molecules (including purification and quality control) is very limited: a procedure taking three half-lives already requires an eightfold amount of radioactive starting material. Together with the negligible mass of even large amounts of radioactivity, this requires extremely fast chemical reactions and procedures. Thus it is just possible to synthesize relatively complicated organic molecules from $^{11}$C for on-the-spot use (three half-lives is 1 h!), but $^{13}$N and $^{15}$O can only be used in simple molecules. $^{15}$O compounds are preferentially made continuously and diverted for intravenous injection as required. The time for the in vivo studies is limited for the same reason.

PET in routine clinical diagnosis

Only a few PET radiopharmaceuticals are used everywhere (15). 2-$[^{18}$F$]$fluorodeoxyglucose ($[^{18}$F$]$FDG), which shows glucose uptake and energy consumption in various cells, is the main PET radiopharmaceutical (8). Amino acid transport and protein synthesis are studied using $^{11}$C- or $^{18}$F-labeled amino acids (5). Blood flow, mainly in heart and brain (but also in other organs), is delineated with $[^{13}$N$]$ammonia or $[^{15}$O$]$water, respectively. But in some institutions, other substances are also used routinely.

$[^{18}$F$]$FDG. The in vivo biochemistry observed with $[^{18}$F$]$FDG is the uptake of glucose in the cell and the corresponding sugar consumption and metabolism. The analogue $[^{18}$F$]$FDG is more apt to show glucose biochemistry than the original compound labeled with $^{11}$C, because the original compound is rapidly oxidized to CO$_2$, which is quickly exhaled. This process is very fast and difficult to register and to interpret by kinetic modeling. $[^{18}$F$]$FDG is taken up by the cells like natural glucose and is phosphorylated by hexokinase in position 1. Further metabolism is now blocked by the fluorine in position 2, and the phosphorylated FDG remains in the tissue. Thus the $[^{18}$F$]$FDG image collected with a PET camera shows regions of high glucose uptake and consumption. In oncology, this enables the identification and localization of many metastases, and the $[^{18}$F$]$FDG image as such is sufficient for diagnosis. Localization of the lesions is improved considerably if the functional PET image is fused with the morphological CT picture (Fig. 2).

In cells with increased energy consumption [e.g., brain, the surviving cells in ischemic heart tissue (17)], sugar is used as the main fuel, because it needs less oxygen to burn than fatty acids. If the myocardial uptake of $[^{18}$F$]$FDG is less than normal, this may be caused by decreased blood flow (e.g., ischemia, the sugar metabolism is still high relative to blood flow but is lower than normal in absolute terms; redress of reperfusion restores the cells) or by a complete blockage of the blood flow (e.g., infarct, in which part of the heart cells are dead). Combination with a myocardial perfusion measurement gives the answer and indicates the way of treatment.

$[^{11}$C$]$- or $[^{18}$F$]$-labeled amino acids. Amino acid transport and protein synthesis may be visualized with labeled amino acids (4, 5, 16). Some tumors, especially in the brain, do not use more glucose ($[^{18}$F$]$FDG) than normal brain tissue but do use excess amino acids. In such cases, labeled amino acids are used for diagnostic purposes in PET, e.g., l-$[^{11}$C$]$methionine or l-$[^{18}$F$]$fluorescein (l-$[^{18}$F$]$FET) (Fig. 3). l-$[^{18}$F$]$FET (14) is not incorporated into proteins, and, because the brain uptake of d-$[^{18}$F$]$FET is negligible, the uptake of l-$[^{18}$F$]$FET is by a

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![FIGURE 1. $[^{18}$F$]$annihilation and the positron emission tomography (PET) coincidence principle.](http://physiologyonline.physiology.org/)

![FIGURE 2. PET-computer tomography (CT) image fusion of a scapula metastasis of a lung carcinoma. The CT picture and the fluorodeoxyglucose (FDG) PET image are shown next to each other. In the lower corner the fused picture is shown. The metastasis was originally not found in the CT, and with PET the exact localization was not possible.](http://physiologyonline.physiology.org/)
specific amino acid transport system. Thus L-[^18F]FET is a marker of specific amino acid transport.

**Biological applications**

In principle, any function in the body could be studied with an appropriately labeled (PET) molecule. In practice, only relatively small molecules, which can be labeled by well-defined organic chemistry methods, are used because of the necessity to (get to) know the physiology and biochemistry of the PET tracer and built in radionuclide. Another prerequisite is that the molecule concentrates in the target organ to such an extent that it is visible over the background. This means that the function to be measured must be concentrated in a well-defined region of interest, an organ or a specific organ region.

In the body, many physiological processes are activated by the binding of an activating molecule (agonist) to a biochemical structure (receptor). The biochemical structure is changed, and this movement initiates a cascade of events. An antagonist binds with high affinity to the receptor but without activation of the physiological process. If the affinity of the antagonist is higher than that of the agonist, the physiological process is (partially) blocked. Receptor-binding substances (ligands) labeled with positron-emitting radionuclides (especially antagonists) can thus be used to visualize and quantify receptors (13).

**FIGURE 3.** L-[^18F]fluoroethyltyrosine (L-[^18F]FET) uptake in astrocytoma. Image was recorded 40-60 min after application of L-[^18F]FET. This type of tumor shows no abnormal sugar uptake (i.e., [18F]FDG PET is not useful).

**FIGURE 4.** Images of N-[11C]methyl-homoepibatidine (HEPB) in the pig brain. Pig brain images of N-[11C]methyl-(−)-HEPB (without and with blockade by cytisine) and of N-[11C]methyl-(+)HEPB are shown. All pictures are coronal brain slices of the same position. Beside the brain, the eyes are visible as two lateral activity spots. The eye uptake is the same in all cases, but the images of the eyes are clearer in the blocking experiment and with N-[11C]methyl-(+)HEPB because the intensity scale is normalized on the most active spots in the image. Only the N-[11C]methyl-(−)HEPB without blocking shows high uptake in the thalamus.
Many receptors are found in the central nervous system, but in the rest of the body many biochemical and physiological processes are also activated by receptors. The identification of the receptors is done by showing that specific molecules bind to these structures. Most receptors are groups of related biochemical structures (subtypes), and the identification of every subtype requires a specific ligand. Ligands labeled with PET isotopes enable in vivo research of the receptors and eventually diagnosis of neurodegenerative (and other) diseases.

Thus we have labeled the enantiomers of epibatidine and homoepibatidine with $^{11}$C (6, 7). These compounds are analogous agonists for a specific subtype of the neuronal nicotinic acetylcholine receptor, which is mainly concentrated in the thalamus. The blockade of the uptake in the thalamus by cytidine, a ligand specific for this subtype, shows the specificity of the binding. Figure 4 illustrates the remarkable selectivity of the receptor: only the (−)-enantiomer binds with high affinity.

**Outlook**

Of rapidly growing interest is the use of PET technology for the development of new drugs (3). Pharmacokinetic studies of new pharmaceuticals are greatly simplified by PET. It is possible to measure the time-dependent biodistribution of a new drug (labeled with a PET isotope) in vivo in one experiment. The effect of a drug on relevant physiological processes (e.g., blood flow, energy metabolism, receptor activation) can be made visible in vivo. Thus the number of animal experiments required to study a new pharmaceutical can be reduced dramatically.

In conclusion, the PET methodology visualizes in vivo biochemical and physiological processes, which opens up fascinating possibilities for basic research and for new, noninvasive medical diagnostics.

**References**