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Physiology 3:230-235, 1988. ;

Physiology (formerly published as News in Physiological Science) publishes brief review articles on major physiological developments. It is published bimonthly in February, April, June, August, October, and December by the American Physiological Society, 9650 Rockville Pike, Bethesda MD 20814-3991.
 ESSN: 1548-9221. Visit our website at http://www.the-aps.org/.

Measurement of Regional Tissue Physiology Using Positron Emission Tomography

Terry Jones

The means for noninvasively studying aspects of regional tissue physiology using positron emission tomography are described. This is illustrated in a review of quantitative methods that have been developed and applied to the study of cerebral, cardiac, pulmonary, and neoplastic tissues.

Introduction

Tracer compounds labelled with γ-ray-emitting radioisotopes have been extensively used to measure entities of regional tissue physiology. Positron emission tomography (PET), although requiring appreciable facilities and resources to implement, is seen as the most advanced methodology for effecting in vivo tracer studies in humans. The purpose of this article is to define the current methodological status of PET for physiological monitoring and to illustrate some of its uses to date.

The PET scanner

The radioisotopes used for PET scanning emit positrons when they undergo radioactive disintegration. These positively charged, electron-like particles are captured by negatively charged electrons. Both particles are annihilated, and two photons, each of 511 keV in energy, are emitted at ~180° to each other. This pattern of emission is used to accurately localise in vivo the distribution of positron-emitting tracers after their administration to a human subject. To record the emergence of these pairs of photons from opposite sides of the body, radiation-sensitive detectors are placed so as to encircle the subject. These recognise, by coincidence circuitry, the simultaneous registration of the two detected γ-rays. If a sufficient number of coincidences and their corresponding angular projections through the body are recorded, then it is possible by computer techniques to reconstruct the transaxial distributions of the tracer within the subject. These are displayed as images representing the tomographic distribution of tracer within the body. To maximise the spatial resolution within the transaxial tomogramme, the aperture of each detector is made as small as practical. To afford axial resolution, a number of these coincidence rings are aligned along the body's length. Figure 1 illustrates a state-of-the-art PET scanner that contains a total of 4,096 individual detector elements, each of which is 5.6 mm wide and 12 mm high. Such a scanner provides, under practical use, a spatial resolution of ~7–8 mm (FWHM) within each of the tomographic transaxial planes and an axial resolution of 7 mm (FWHM). This array of detectors provides for the continuous recording of tracer within a 10-cm axial field of view. This means, for example, that the whole of the human myocardium or most of the human brain can be continuously monitored and hence the time courses of their regional radioactive content followed. The spatial and temporal resolutions of a PET scanner are complemented by the ability to convert the picture element counts within the reconstructed tomogrammes into absolute units of the tissue's radioactive content. This follows, since it is possible to correct for perturbations in the recorded counts caused by some absorption of emitted γ-rays in the body's tissues.

Positron-emitting radioisotopes

The spatial, temporal, and quantitative accuracies of the PET scanner are matched by the biological compatibility of the positron-emitting radioisotopes that are used for the labelling of tracer molecules. Principal among these are oxygen-15, nitrogen-13, and carbon-11 which, although having half-lives of 2.1, 10, and 20.1 min, respectively, are the longest lived γ-ray-emitting forms of these basic biological elements. There is no suitable positron-emitting isotope of hydrogen but fluorine-18, with a 110-min half-life, is used as a labelling substitute. These short-lived isotopes need to be manufactured close to the site of their application, and this is usually achieved with a particle accelerator such as a cyclotron. It can be seen that with these radioisotopes, the scope for labelling biomolecules and pharmaceuticals is immense. However, to realise this, innovative and fast radiochemical procedures are needed to provide enough labelled compound to record meaningful tomogrammes with the PET scanner. Such specialised radiochemistry is already well advanced. Synthetic procedures have been identified whereby any one of ~80% of the compounds contained in the pharmacopoeia could be labelled with carbon-11 and purified for administration to humans in ~40 min of chemistry after this isotope's production.

Tracer kinetic models for physiological measurement

To obtain quantitative values of physiological entities using tracers it is of course necessary to implement tracer kinetic models that describe

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0886-1714/88 $1.50 © 1988 Int. Union Physiol. Sci./Am. Physiol. Soc.

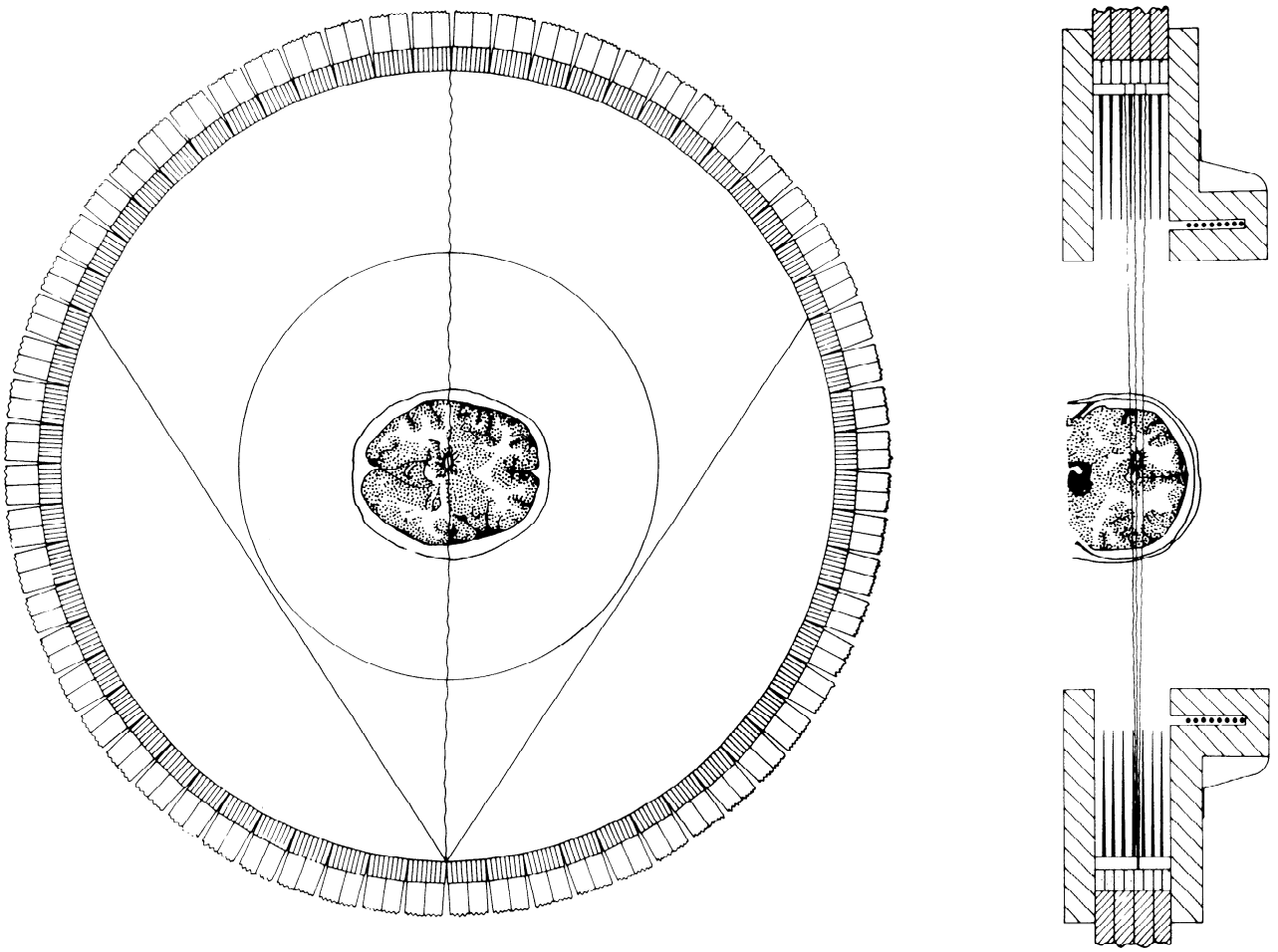

FIGURE 1. Physical arrangement of modern positron emission tomography scanner that is comprised of ~4,096 individual detector elements. These are arranged in 8 continuous rings of coincident detectors that can record and reconstruct tomographic data of distribution of positron-emitting isotopes within the human body.

the biological fate of the markers. The scan data are used to solve the respective kinetic equations to derive tomographic values of the physiological parameter of interest. In such models, the tissue's content of tracer invariably needs to be expressed in terms of either the concentration of the isotope in the blood and its time course or the amount of radioactivity administered. It is here that the quantitative accuracy of the PET scanner is important. This follows, since the picture element response within the reconstructed tomogrammes can be calibrated against the response of a well counter used to measure withdrawn blood samples or aliquots of the total amount of radioactivity administered. If a sufficiently large vascular volume is contained within the field of view, as, for example, in the case of cardiac studies, then the blood concentration and the time course can be obtained directly from within the scan data. For extended reading of PET methodology, Ref. 9 provides a broad basis. Despite the technical advantages that PET has for collecting data that can be processed to solve tracer models, the quantitative PET procedures developed to date are comparatively few. In effect, the technology of PET has advanced further than the ability to interpret the recorded data. In the following sections, examples are given of where the methodology has been developed to provide quantitative entities of regional tissue function in humans. For this I have considered those organs and tissues that have been the focus of most attention.

Brain

The human brain has to date been the principal organ of interest for use of PET. The highly structured physical distribution of function within the cerebral tissues maximises the use of PET's spatial power. From the physiological standpoint, emphasis has been placed on relationships between nutrient supply (blood flow) and utilisation (energy metabolism). In addition, haemodynamic relationships such as the ratio of blood flow to blood volume have been explored together with blood-brain-barrier transport and components within neurotransmitter pathways.

Methods established to measure regional cerebral blood flow using PET have focussed principally on the use of oxygen-15-labelled water, although labelled methane has also been used. The water studies have involved either intravenous injection of $H_2{}^{15}O$ or the inhalation of tracer amounts of CO_2 labelled with ^{15}O. The latter procedure results in a shift of the label from the CO_2 to the water pool within the lung tissue. This provides an input of la-

belled water into the arterial tree. The earlier, slower generation of PET scanners saw the introduction of a steady-state administration of $H_2{}^{15}O$ and the recording of tomographic data under conditions of dynamic equilibrium. The faster scanners enabled more transit measurements to be carried out, either by bolus administration or by buildup measurements during a continuous administration over a 2- to 3-min period. In all cases, arterial blood samples are withdrawn. These are measured either in a well counter or continuously with an on-line detector viewing the catheter through which arterial blood is continuously withdrawn. The regional values of cerebral blood flow are computed as milliliter per milliliter per minute. The tracer kinetic models for measuring flow were based on the simple Fick principle in which a term for the relative distribution volume of the label within the tissues has to be included. An average in vitro measured value for the ratio of brain tissue to blood partitioning has been used. Currently effort is focussed on including this as a fitting term in the kinetic equations in order to account for it specifically for the tissue in question. It remains to be seen if this can be included systematically and reliably in the processing of human scan data. Values of cerebral blood flow by themselves have found limited application, since they have been shown to be more variable than measurements of the cerebral tissue's metabolic rate. This means that the coupling between flow and the fractional extraction of a metabolic substrate is variable, whereas the metabolic rate is kept constant. PET measurements of cerebral blood flow have been used for brain activation studies. A review by Roland (10) in a previous issue of *NIPS* covers this, and recent studies have shown new information on how regional cerebral physiology responds to activation (7). The short 2.1-min half-life of oxygen-15 makes it possible to repeat studies over a time period that is acceptable for human investigation. This area promises to be of interest in neurological or psychiatric conditions in which the normal physiological response to activation may be altered. There is a continuing need for measurements of cerebral blood flow in order to interpret the uptake and retention of labelled metabolic substrates or pharmaceuticals.

Regional cerebral metabolic rate. Because oxygen and glucose are the brain's principle energy substrates, PET methods have focussed on measurements of their regional cerebral utilisation rate. From the kinetic point of view, measurements of oxygen consumption have associated with them less modelling assumptions. Here the fate of the oxygen is metabolic water, and simple kinetic models include terms for blood flow and the fractional arteriovenous extraction of oxygen. An additional component representing the presence of oxygen-15 contained within the vasculature has also been included. Similar to the blood flow methods, two approaches have been developed. One involves the continuous inhalation of oxygen-15 with data collected under steady-state conditions. The procedure is followed by the above steady-state measurement of blood flow. From the two studies, values for the regional tissue's fractional extraction of oxygen (arteriovenous difference) can be derived. The product of extraction, blood flow, and the arterial content of oxygen provide an absolute value of the regional cerebral metabolic rate for oxygen (ml $O_2 \cdot ml^{-1} \cdot min^{-1}$). For accuracy, a third procedure is introduced that involves the subject inhaling trace amounts of CO labelled either with oxygen-15 or carbon-11. From the corresponding scan data and blood samples, regional cerebral blood volume can be obtained (ml/ml) and the radioactivity present in the vasculature during ^{15}O inhalation can be accounted for. The above sequence of measurement has also been carried out dynamically involving a few breaths of ^{15}O for oxygen uptake and the injection of $H_2{}^{15}O$ for blood flow. More recently, a single procedure was reported involving only the inhalation of ^{15}O and fitting the kinetic data to obtain values for the cerebral metabolic rate for oxygen (6). Included in this fit are terms for blood flow and the vascular component. When more experience is gained with this single procedure, rapid metabolic activation studies of regional human brain promise to become a reality.

The measurement of regional cerebral glucose utilisation has relied mainly on the use of the analogue deoxyglucose labelled with fluorine-18. The metabolic trapping of this tracer provides a definable tracer model and procedure. Glucose utilisation is obtained either from the rate of uptake of the analogue or the amount of activity accumulated at a period of time ($mg \cdot ml^{-1} \cdot min^{-1}$). In both cases, the blood concentration of tracer has to be measured in parallel to PET scanning. Some questions arise as to the relative affinity of the tissue's utilisation of deoxyglucose compared with that of natural glucose. Although this can be measured for normal cerebral tissue, there is concern that it may be different for a disease state. Natural glucose, uniformly labelled with carbon-11, has also been tried. However, the rapid metabolism of glucose and release of $^{11}CO_2$ together with variable transitions into other metabolic pathways make the kinetic modelling of this tracer tenuous. More recently, efforts have gone into selectively labelling the glucose with carbon-11 in the one or six position. Here the aim is to provide for more time before the loss of the metabolic products. It remains to be seen how useful this approach will prove to be.

^{18}F-labelled deoxyglucose has been used for regional cerebral activation studies. However, many cerebral responses are rapid and subtle and require, in the same subject, repeat test-to-control comparisons to achieve statistical significance. Here the ^{18}F-deoxyglucose method with the 110-min half-life isotopes is not as practical as oxygen-15 blood flow or utilisation studies. To help overcome this, use has been made of [^{11}C]deoxyglucose.

The principal human use of oxygen-15 and ^{18}F-deoxyglucose for measurements of cerebral metabolism have been in neurological and psychiatric research. The brevity of this review does not allow a discussion of the clinical research results to date. However, experience has shown that studies involving combined measurements of oxygen utilisation and blood flow or glucose utilisation have defined pathophysiological profiles that would not

have been provided from any one single meausurement. Indeed the investigative power of PET stems to a large extent from being able to approach a pathological state from different tracer pathways, thereby helping to piece together the underlying disturbances in tissue functions. A simple example of this is the study of patients shortly after suffering an acute stroke. Here it has been shown that a state of mismatching between the normally coupled cerebral blood flow and oxygen utilisation rate can occur. This defines the tissue's response to the lesion and possible mechanisms for its survival.

Cerebral haemodynamics. One physiological entity that has been of interest in cerebral vascular disease is the relationship between regional cerebral blood flow and blood volume. In the presence of falling perfusion pressures, brought about through carotid stenosis, cerebral blood flow appears to be maintained through vasodilation, which manifests itself as an increase in regional cerebral blood volume.

Blood-brain barrier. Active transport across the blood-brain barrier has been studied regionally in the human brain using PET scan data and the time course of activity in the arterial blood. An example of this is the study of [^{11}C]methylglucose, which is transported but not metabolised and has been used to investigate physiological changes to the barrier in chronic diabetes. Leakage of the barrier has been studied using the cation rubidium-82 (78-s half-life)- and gallium-68 (68-min half-life)-labelled EDTA. When combined with a measure of regional cerebral blood flow, measurements of the barrier can be obtained as a permeability-surface area product.

Neurotransmitters and receptors. There currently exists much interest in using PET with labelled precursors and neurotransmitter systems and labelled ligands to delineate cerebral receptors. Most advances have been made into the dopaminergic system with ^{18}F-labelled L-dopa being used to define the synthesis of dopamine. Various ligands such as [^{11}C]raclopride, [*methyl*-^{11}C]spiperone, and ^{18}F-spiperone delineate the D_2 postsynaptic receptors and [^{11}C]nomifensine delineates the presynaptic reuptake site (11). Already these dopaminergic markers have formed an armamentarium to study patients with movement disorders, and again the combined use of selective markers has helped to describe the underlying pathophysiologies. Other systems successfully studied with PET include diazapine and opiate receptors (2), while much effort is currently focussed on seeking suitable markers for the serotonergic pathways. References 5 and 8 provided extensive reviews on the use of PET for cerebral studies.

Heart

The second most intensive area for PET application to date has been in the myocardium with a particular emphasis on coronary artery disease. Here the concentration has been on recording representations of regional myocardial blood flow and, where possible, relating these to myocardial metabolism.

Myocardial blood flow. Measurements of regional myocardial blood flow with PET are not as advanced as flow methods for the brain. The use of inert diffusible tracers such as $H_2{}^{15}O$ are hindered by the juxtaposition of the cardiac chambers and cross talk of tracer contained therein onto the recording of activity within the muscle wall. The initial approach to measuring flow was to use cationlike tracers such as radioisotopes of rubidium (^{82}Rb), potassium (^{38}K), and ^{13}N-labelled ammonia. The high first-passage extraction and retension of these tracers in the myocardium provide an index of muscle blood flow once the blood level has decreased. Rubidium-82 with its 78-s half-life has been useful for rapid repeat measurement of myocardial flow in transit ischaemic states. The advent of faster PET scanners with higher spatial resolution has seen a focus once again on the purer flow marker $H_2{}^{15}O$. Here, in the developed kinetic model, terms are included for cross talk from the cardiac cavities. This is made possible by delineating the time course of activity in the cavities themselves. Promising reports are emerging that demonstrate that regional cardiac muscle blood flow, the physiological parameter most central to the manifestation of myocardial ischaemia, can be quantitated in humans by use of PET.

Myocardial metabolism. The goal for measuring myocardial metabolism is to relate it to corresponding measurements of blood flow, thereby defining states of tissue ischaemia. The first focus of attention was to use ^{11}C-labelled fatty acids. Labelled palmitate has proved interesting but [^{11}C]acetate provides for a simpler approach to define β-oxidation. Even with this tracer, it has not been possible to date to formulate a quantitative kinetic model. The success of the use of ^{18}F-deoxyglucose in the brain has led to its use in the myocardium, not the least because a definable kinetic model is available. To date, comparisons between myocardial glucose utilisation and indices of muscle blood flow using rubidium-82, potassium-38, or $^{13}NH_3$ have provided interesting information on the pathophysiology of coronary artery disease. The myocardium recovering from a transit ischaemic event was shown to increase its transport of glucose. Patients with chronic states of unstable angina also show a higher uptake of glucose, and in those patients recovering from a myocardial infarction, an increase of glucose transport (^{18}F-deoxyglucose) into the muscle relative to the blood flow ($^{13}NH_3$) is indicative of a good prognostic outcome (3, 12).

The use of PET to study neurotransmittor systems in the myocardium is less advanced than in the brain. The exceptions to this are the studies of the muscarinic receptor using [^{11}C]MQMB and the β-receptor using [^{11}C]CGP 12177 (3).

Lung

Regional pulmonary physiology studies using PET have been confined to comparatively few centers. Nevertheless, methods have been developed to measure tomographically a number of physiological entities that are of traditional interest in the study of the lung's role in gas exchange. Measurements have been made of regional lung density (g/ml of thorax) using PET transmission scans and blood volume (ml/ml of thorax) obtained with ^{11}CO-labelled red blood cells. The scaled quantitative subtraction of these parameters provided tomographic measurements of regional extravascular lung density (g/ml of thorax). These have

been used to study the distribution of pulmonary oedema in both acute and chronic cardiac disease. The difference between PET scans of $H_2{}^{15}O$ and ^{11}CO-red blood cells has been used as a measure of exchangeable extravascular water in the lung. The subtraction of the values of regional lung density from unity has been used as a measure of regional lung alveolar volume (ml/ml of thorax). The ratio of this to regional extravascular density provides indices of regional alveolar size (ml/g of lung tissue) in normal subjects. It has been of interest to compute the ratio between regional alveolar size and blood volume. This has been shown to decrease in the gravity-dependent areas of the lung where gas exchange is most efficient.

Tomographic measurements of regional lung ventilation have been possible by the continuous inhalation of neon-19 (17.3-s half-life). A simple steady-state kinetic model has been used that expresses regional ventilation in terms of the inspired and lung concentrations of tracer. The solution of this model, using the recorded data, provides ventilation in milliliter per minute per milliliter of alveolar volume. By use of the additional measurements of gas volume and extravascular density, values of ventilation per unit volume of the thorax and per alveolus can be calculated. Tomographic measurements of the ratio between regional lung ventilation and blood perfusion are possible using the continuous intravenous infusion of ^{13}N solution. Here the steady-state pulmonary concentration of tracer relative to that within the right heart chambers represents the balance between the isotope's delivery to the lung alveoli and its removal by ventilation. By combining this ventilation-to-perfusion measurement with that of ventilation alone (^{19}Ne), it has been possible to measure regional pulmonary perfusion per alveolus. These entities, which contribute to pulmonary gas exchange, were used to study smokers and a range of respiratory diseases including asthma and chronic obstructive lung disease. Serial measurements of regional lung red blood cell volume (^{11}CO-red blood cells) and plasma volume ([^{11}C]albumin) provided values of regional lung haematocrit.

The lung tissue's rate of energy metabolism is comparatively low. Nevertheless, it has been possible to measure regional lung glucose utilisation using ^{18}F-deoxyglucose. The sequestration of this glucose analogue makes possible a measurement of pulmonary metabolism that is otherwise impossible from attempted measurements of the small arteriovenous differences of natural glucose across the lung. ^{18}F-deoxyglucose has been used clinically to study interstitial lung disease. In sarcoidosis, increased focal accumulations of tracer were found and ascribed to the infiltration of inflammatory cells that are known to metabolise glucose anaerobically.

Future use of PET scanning in lungs is destined to focus on the sequestration of labelled serotonin as a marker of pulmonary endothelial function. Also there is a prospect of using labelled ligands to research the role of alveolar β-receptors in pulmonary diseases. Reviews of PET lung studies are found in Refs. 3 and 4.

Tumours

There is a growing interest in using PET to study the pathophysiology of human solid tumours. To date, research has centred on the measurement of tumour blood flow and glucose and oxygen utilisation using methods developed for brain studies. Some uncertainty exists with respect to extrapolating the use of kinetic models developed and validated for cerebral tissue for studying the pathophysiology that exists in tumours. Results to date have confirmed the glycolytic nature of neoplasms, and some centres use this to detect the regrowth of tumours after therapy. The relationship between tumour blood flow and oxygen utilisation has shown that the bulk of a tumour's blood flow is associated with a comparatively low fractional extraction of oxygen (small arteriovenous O_2 difference). This presumably reflects a tumour's preference for glycolysis and has been confirmed from studies of brain tumours where the ratio of oxygen utilisation to glucose utilisation is low compared with that of normal surrounding brain. It needs to be emphasized that the recorded signals are weighted to the well-perfused areas of the tumour. This may mask the high fractional extraction of oxygen that is believed to occur in the peripheral, poorly perfused areas of the tumour, resulting in tissue anoxia.

The search continues for markers of tumour proliferation and here labelled amino acids have been used with PET to delineate protein synthesis. More recently ^{18}F-labelled deoxyuridine and [^{11}C]thymidine were used to examine DNA synthesis of human tumours. Other pioneering studies include the use of labelled ligands to delineate receptor activities in breast cancer, and labelled polyamines such as [^{11}C]putrescine have been shown to be highly sequested in brain tumours. Labelled chemotherapeutic agents have also been used to measure drug delivery, concentration, and time course in human neoplastic tissue. Reviews of PET tumour studies are contained in Refs. 1 and 3.

Reference 3 contains recent reviews of the clinical efficacy of PET.

In this review the facilities and resources necessary to implement physiological measurements using PET are only discussed in outline. Also, no mention has been made of the logistical difficulties of coordinating the multi-disciplinary effort needed to effect innovative programmes of study. These are further complicated by the pressure of time imposed by the rapid decay of the radiotracers used. Nevertheless, it is important to stand back from these practicalities and realise that, similar to any other area of investigation, it is the research questions being asked and the ability to interpret the recorded data in scientific terms that inevitably become the most important issues. Many centres are still at the stage of formulating the conditions necessary to collect PET data. Among these is the need to handle the vast amount of spatial and temporal data that modern PET scanners are capable of collecting. Currently much emphasis is being placed on computer graphics techniques to condense these data and present them in a form that can be comprehended and appreciated by the scientific community.

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Two Interacting Signal Transduction Pathways Regulate Collecting Duct Water Transport

Yasuhiro Ando, Harry R. Jacobson, and Matthew D. Breyer

Receptor-mediated signal transduction occurs through phosphatidylinositol bisphosphate (PIP_2) breakdown and activation of adenylate cyclase interacting to regulate cell function. Current studies suggest that hormone-stimulated PIP_2 breakdown modulates the classic cyclic AMP-mediated hydrosmotic action of vasopressin through separate mechanisms attributable to activation of protein kinase C, elevation of intracellular Ca^{2+} concentration, and generation of arachidonate metabolites.

Introduction

The receptor-mediated breakdown of phosphatidylinositol bisphosphate (PIP_2), a minor constituent of cell membrane phospholipids, is employed by many biologically active agents and hormones to initiate two major intracellular signal transduction events: the activation of protein kinase C (PKC) and the mobilization of Ca^{2+} from intracellular storage pools. As is now well known, two products of phospholipase C-mediated PIP_2 hydrolysis, diacylglycerol (DAG) and inositol triphosphate (IP_3), are responsible for activation of PKC and elevation of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), respectively (15). Furthermore, the latter results in activation of Ca^{2+}-calmodulin-dependent protein kinase. Phosphorylation of substrate proteins by activated protein kinases is believed to be essential to complete signal transduction. During this signaling process, various biologically active metabolites of arachidonic acid (AA), e.g., prostaglandins, can be generated from the AA released from DAG or its metabolite phosphatidic acid or from the AA released by the Ca^{2+}- or PKC-activated phospholipase A_2 (11, 15).[1]

The PIP_2 breakdown-initiated signaling system has been universally documented in all mammalian cell types so far examined. When measured in a cell-free system with histone used as a common substrate, the total activity of PKC is higher than that of adenosine 3′,5′-cyclic monophosphate (cAMP)-dependent protein kinase (or protein kinase A) in most organs, including the kidney

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[1] Although PIP_2 breakdown has been so far regarded as a prerequisite for DAG formation and PKC activation in intact cells, several mechanisms for PIP_2-independent PKC activation have been reported to exist in certain cells (e.g., Farese et al., *Science Wash. DC* 236: 586, 1987; Besterman et al., *Proc. Natl. Acad. Sci. USA* 83: 6785, 1986; Peter-Riesch et al., *J. Clin. Invest.* 81: 1154, 1988. Also, needless to say, IP_3 formation is not the only way to increase $(Ca^{2+})_i$, e.g., in hepatocytes, cAMP increases $(Ca^{2+})_i$ by stimulating Ca^{2+} influx. Likewise phospholipase A_2 can be activated more directly via a receptor-coupled mechanism, e.g., bradykinin stimulation of prostaglandin synthesis in fibroblasts.

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