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POSITRON EMISSION TOMOGRAPHY IN ASSESSMENT OF REGIONAL STEREOSPECIFICITY OF DRUGS

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Abstract—PET imaging has much to offer the pharmaceutical industry in the design and development of drugs, beginning with the radiolabelling of drugs to facilitate pharmacokinetic studies and extending to measurement of the effects of drugs on regional metabolism, such as that of glucose and oxygen, or in stimulating or blocking receptors, occupying enzymes or affecting substrate transport processes.

Positron emission tomography (PET) is the quantitative imaging of the distribution of positron-emitting radiotracers within various organs of the body after their intravenous injection [1, 2]. PET can help in the design and development of drugs by making it possible to measure regional concentrations of chemical substances *in vivo* with almost the same sensitivity and specificity that radioimmunoassay makes possible in the measurement of chemical substances in body fluids.

PET has opened a new era in human biochemistry, and promises to have several important applications in pharmacology and drug development. Applications of positron emission tomography in pharmacology include the radiolabelling of drugs themselves, and of radiotracers, such as labelled glucose and oxygen, that make it possible to assess the effects of drugs on regional substrate metabolism; or to assess the pharmacodynamics of drugs on recognition sites in various organs of living human beings.

In vivo pharmacokinetics can be measured in organs such as the liver, kidneys and brain, supplementing the data available from pharmacokinetic studies of body fluids, such as plasma and urine. PET can be of particular value in assessing stereoselective binding of enantiomers in various organs, providing data of importance in the design and development of effective drugs with a minimum of toxic side effects. Many synthetic drugs are administered as racemic mixtures, often with their effectiveness and toxicity resulting from the binding of enantiomers by different binding sites.

Quantitative measurement by PET of the effects of drugs on regional glucose or oxygen metabolism makes it possible to determine the site and quantify the action of the individual enantiomers, as well as the racemates, just as one can, with PET, compare the pharmacokinetics of the racemates and their enantiomers.

PET is well suited for quantification of the availability and affinity of recognition sites, making it possible to examine directly in the various organs of living human beings the effects of drugs that act by blocking (antagonists) or stimulating (agonists) such recognition sites, including those that bind classical neuroreceptors or peptide hormones, or are enzymes involved in substrate transport processes. Species differences in the metabolism of racemates and enantiomers are additional reasons why studies of human beings by PET might provide important information in human pharmacology.

EXAMPLES

PET has been used in assessment of the effects of drugs that bind to dopamine or opiate receptors, as well as in the study of drugs, such as carbon-11 deprenyl, that are bound by enzymes, in this case, monoamine oxidase B [3–5]. Figure 1 shows the first images of a neuroreceptor obtained by positron-emission tomography in a living human being, a study performed on 25 May, 1983 [6]. The yellow regions in the center of the PET image show the high degree of binding of the positron-emitting radiotracer, carbon-11 N-methyl spiperone, by the D2 dopamine receptors in the caudate nucleus and putamen. Figure 2 illustrates the differences in binding of carbon-11 N-methyl spiperone, which binds to D2 dopamine receptors, and carbon-11 carfentanil, a narcotic that binds to mu-type opiate receptors [7, 8].

Quantitative PET imaging makes it possible to determine the relationship between the dose of neuroleptic drugs, the degree of occupancy of dopamine receptors, and the behavioral responses to the drug. The effects of antidepressant drugs, such as deprenyl, in occupying the enzyme, monoamine oxidase, can be related to the clinical response of the patient.

DOSE/RESPONSE MEASUREMENTS

Dose–effect relationships can be examined directly. For example, Fig. 3 shows the complete blocking of the accumulation of carbon-11 carfentanil by mu-type opiate receptors in three different cross-sectional slices of the living human brain. In the upper row, the tracer can be seen to have accumulated in the temporal lobes and pituitary (far left image), in the frontal and parietal cortex, caudate nucleus, putamen and medial thalamus (middle image); and in the upper cortical regions except for the sensory-motor cortex (far right). In the middle image (upper row), it can be seen that the visual cortex does not bind the tracer, indicating the lack of mu-type opiate receptors in this region. The lower

row of images were obtained in a second study in which an intravenous dose of 1 mg/kilo of naloxone was injected prior to a second injection of carbon-11 carfentanil, and completely blocked the accumulation of the carbon-11 carfentanil. Figure 4 illustrates the effect of a smaller dose of naloxone in decreasing the accumulation of the tracer carbon-11 carfentanil, but to a lesser degree than that produced by a large dose. One can obtain a dose–response curve to show the different degrees of binding of different doses of naloxone to opiate receptors in the living human brain [9]. As the administered dose of naloxone was increased, there was a progressive decrease in the ratio of the accumulation of carbon-11 carfentanil in the brain as represented by the ratio of binding without and with the prior administration of the various doses of naloxone. To perform this type of study, one does not always need a PET scanner, that is, a high degree of spatial resolution may not be needed in the measurements. As can be seen in Fig. 3, naloxone occupies the mu-type opiate receptors throughout the brain in a similar fashion. The simple dual detector system illustrated in Fig. 6 is much less expensive than a PET scanner and requires only 1/50th the amount of administered tracer [10]. This makes possible multiple studies of the effects of drugs in the same person at low cost and at low radiation dose to the subject. As shown in Fig. 7, the effect of naloxone in releasing the previously accumulated carbon-11 carfentanil from the brain can be measured. The upper curve in Fig. 7 is the time course of the radioactivity within the brain after an injection of carbon-11 carfentanil in the control state. Administration of naloxone 15 min after the injection of the carbon-11 carfentanil at the time indicated by the arrow resulted in a release of the carbon-11 carfentanil from the receptors, indicated by the fall in measured radioactivity.

In an analogous study illustrated in Fig. 9, one can see the effect of administering an oral dose of haloperidol 4 hr before the injection of carbon-11 *N*-methyl spiperone. The image on the left shows the accumulation of the tracer 45 min after injection in the control state, while the image on the right shows that the administration of haloperidol inhibited the accumulation of the same dose of carbon-11 *N*-methyl spiperone that had been given in the control study.

Figure 10 illustrates the accumulation of carbon-11 *N*-methyl spiperone by D2 dopamine receptors, together with an image showing the accumulation of the experimental drug SCH 23390 than binds to D1 dopamine receptors.

All aspects of dopamine metabolism can be measured by PET, beginning with the synthesis of dopamine from L-DOPA [11]. In some neurons, dopamine is a precursor of norepinephrine. In those neurons which utilize dopamine as a neurotransmitter, dopamine is secreted into the synapse and binds to dopamine receptors on the membranes of post-synaptic neurons [12]. PET makes it possible to quantify the concentration of receptors in regions of the brain, such as the caudate nucleus and putamen, that are rich in dopamine receptors [13, 14]. With the appropriate radioligand, one can measure the secretion of the endogenous neurotransmitter by its competition with the radioligand for binding to the receptor.

Unbound dopamine within the synapse is also bound by autoreceptors on the presynaptic neurons or metabolized by the enzyme, monoamine oxidase B. Both processes can be measured by PET. Stereospecificity is exemplified by the fact that L-deprenyl is bound by sites containing monoamine oxidase to a degree 25 times that of D-deprenyl [4].

PET makes it possible to assess the pharmacokinetics of labeled drugs, whether administered as racemates or in enantiomeric form; to measure regional metabolic effects of the enantiomers on various organs of the living body; and their binding to recognition sites.

The spatial resolution of such studies at present is 4.5 mm [15], as illustrated in Fig. 11, which shows multiple images of glucose metabolism in a normal person, following a 10 mCi dose of fluorine-18 2-*d*-deoxyglucose. These images were obtained by a state-of-the-art Japanese PET scanner, and are shown with permission [16].

Increased neuronal activity in various brain regions can be measured as increases in regional blood flow or substrate metabolism, using either glucose or oxygen, or by measuring the secretion of neurotransmitters.

An example of the use of fluorine-18 deoxyglucose to assess the regional effects of the administration of drugs is the study of London *et al.* [17]. Morphine administration to human beings under controlled conditions brought about a decrease in glucose metabolism in certain brain regions, especially the superior and medial frontal gyri.

DURATION OF ACTION

Another example of the use of positron emitting radiotracers in pharmacology is the measurement of the duration of action of naltrexone, a long acting antagonist of opiate receptors. Naltrexone is used in the treatment of opioid dependence. The drug has a longer duration of action than naloxone, which is used to treat acute overdosage of opiates. The apparent plasma half-time of naltrexone is approximately 4 hr, while that of its major active metabolite, beta-naltrexol, is 12 hr after an oral dose [18, 19]. Using a simple dual detector system for measuring the rate of accumulation of the radioactive narcotic, carbon-11 carfentanil, it was possible to measure the degree and duration of occupancy of mu-opiate receptors in the brain of normal human volunteers after a single 50 mg oral dose of naltrexone [20]. The binding of carbon-11 carfentanil in the brain of normal volunteers was measured with the dual-detector system before and 1, 48, 72, 120 and 168 hr after a single oral dose of 50 mg of naltrexone. The half-time of duration of blockade by naltrexone in the brain ranged from 72 to 108 hr, which is greater than the fast components of clearance of the drug and its principal metabolite, naltrexol, from plasma.

Verebey *et al.* have reported that in addition to the two initial components of naltrexone clearance, there is a third phase with an estimated plasma clearance half-time of 96 hr [21]. Verebey found that

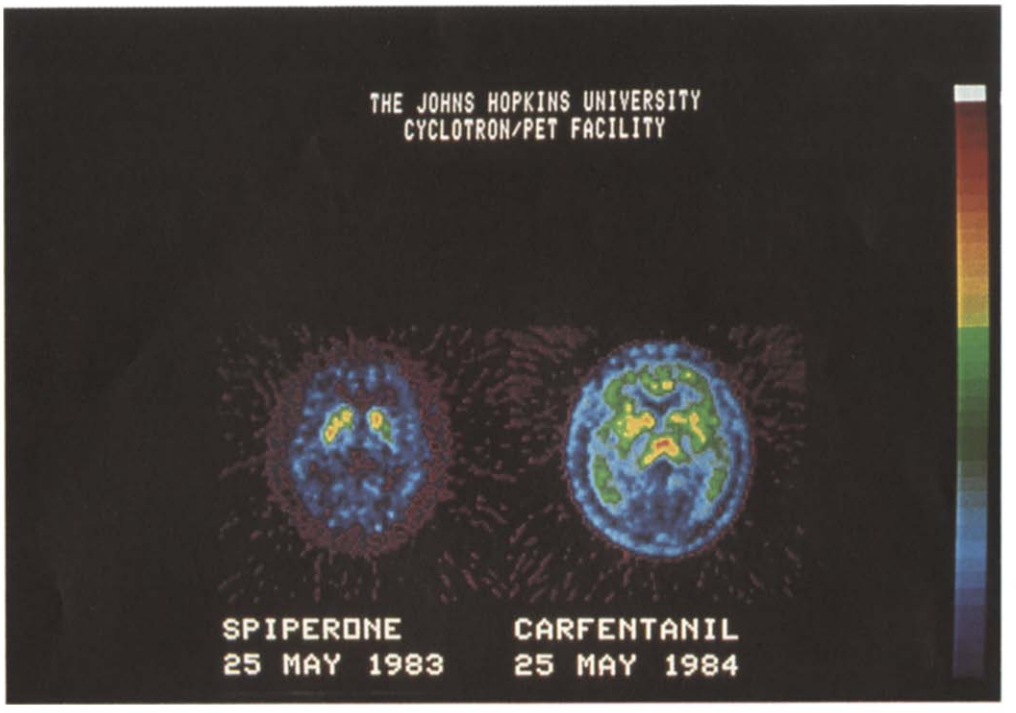

Fig. 1. First imaging of a neuroreceptor in a living human being. The tracer was carbon-11 *N*-methyl spiperone that binds to D2 dopamine receptors, indicated by the yellow regions that correspond to the caudate nucleus and putamen, which contain over 90% of the dopamine receptors in the brain.

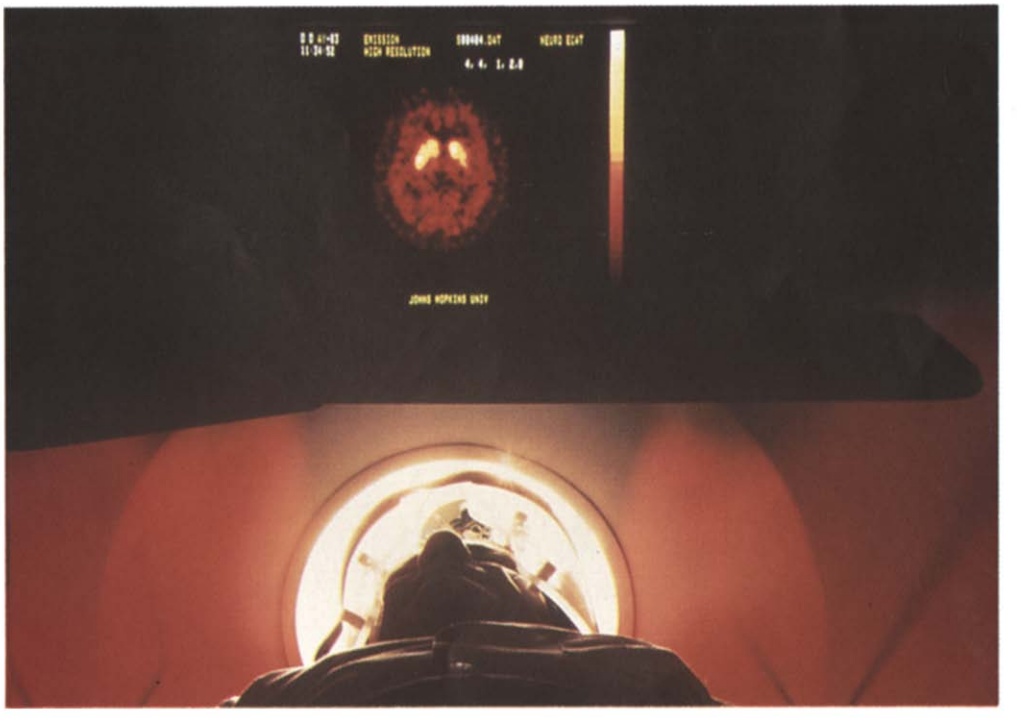

Fig. 2. Left: images of D2 dopamine receptor binding of carbon-11 *N*-methyl spiperone; right: images of the distribution of mu-type opiate receptors in the human brain imaged by the administration of carbon-11 carfentanil, a narcotic that is nearly 10,000 times more potent than morphine.

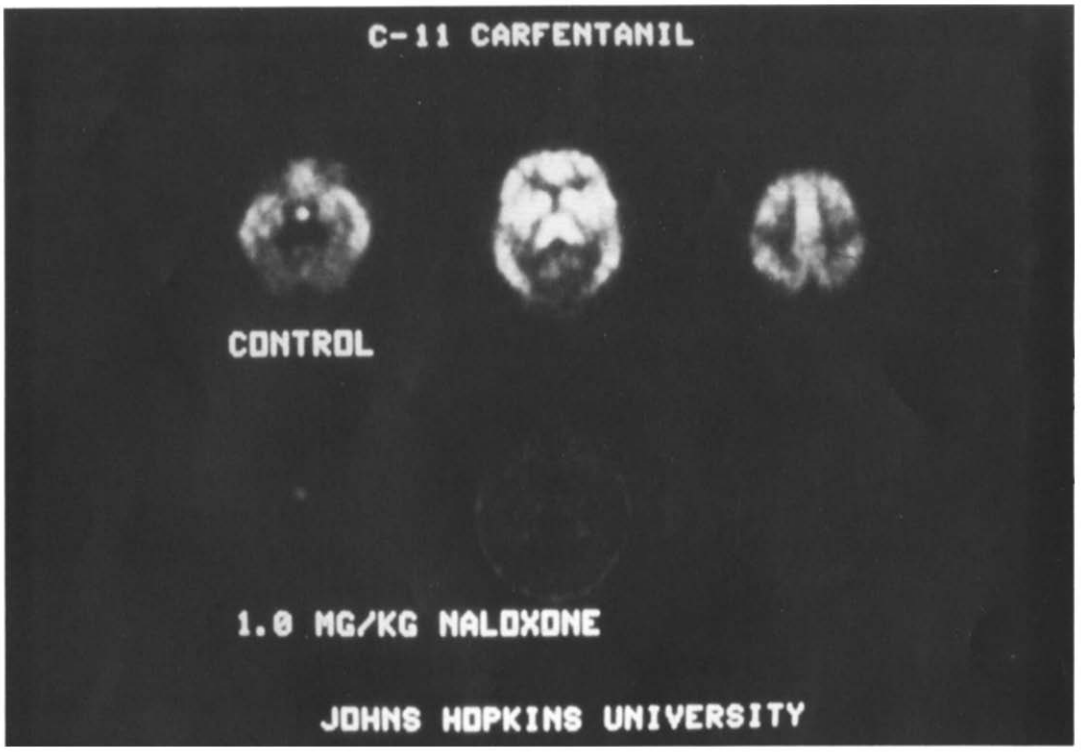

Fig. 3. Upper row: binding of carbon-11 carfentanil to opiate receptors in three cross sectional images of the human brain. The left image reveals the receptors in the pituitary and temporal tobes; the middle slice, the receptors in the frontal and parietal cortex, the caudate nucleus and putamen, and the media thalamus; the right image shows the receptors in the cortex, except for the sensorimotor cortex. The lower row of images indicate the blocking of the receptors by the prior administration of naloxone.

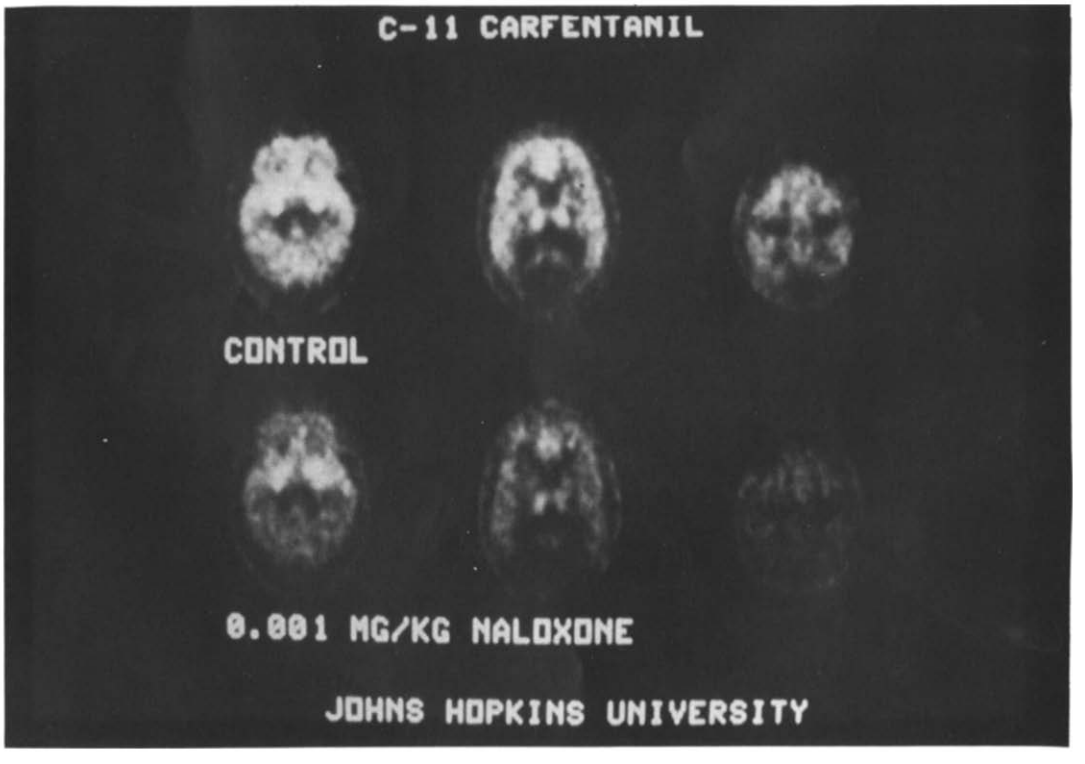

Fig. 4. Partial blockade of the receptors by the administration of a lower dose of naloxone than that used in Fig. 3.

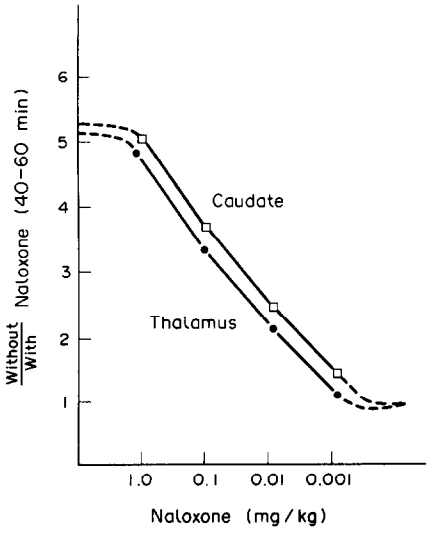

Fig. 5. Decrease in receptor binding of carbon-11 carfentanil produced by increasing doses of naloxone, i.e. a dose response curve in a human being.

inhibition of the physiologic and subjective effects of heroin in human volunteers persists for 72 hr after 100 mg of oral naltrexone, and concluded that 2.4 ng/ml of plasma naltrexone was high enough to completely antagonize the effects of 25 mg of heroin 48 hr after 100 mg of oral naltrexone.

The duration of receptor occupancy by naltrexone measured with carbon-11 carfentanil was similar to the duration of the pharmacological effects of naltrexone measured by a heroin challenge, and correlated well with the long half-time of the terminal phase of the plasma clearance of naltrexone (96 hr). These results indicate that 50 mg of oral naltrexone results in plasma levels of naltrexone and beta-naltrexol far greater than that needed to totally occupy opiate receptors. The half-time of the third plasma component (96 hr) which was observed after plasma levels had fallen to 2.4 ng/ml, corresponds to the half-time for reappearance of unoccupied opiate receptors (72–108 hr). Doses lower than the recommended 50 mg/day of oral naltrexone should result in complete occupancy of opiate receptors, and perhaps be associated with fewer side effects.

SCHIZOPHRENIA

With high affinity, essentially irreversibly bound radiotracers, such as carbon-11 *N*-methyl spiperone, the endogenously secreted neurotransmitters, such as dopamine, are not able to compete successfully for binding to the receptor, which is an advantage in measuring receptor density, but if one wishes to study factors that bring about secretion of endogenous neurotransmitters, one must use tracers with a lesser affinity, so that the endogenous neurotransmitter will compete with the radiotracer for binding by the receptor [3, 13, 14].

D2 dopamine receptors have been found to be increased in the caudate nucleus and putamen of some schizophrenic patients who had never been treated with neuroleptic drugs [22]. Some patients

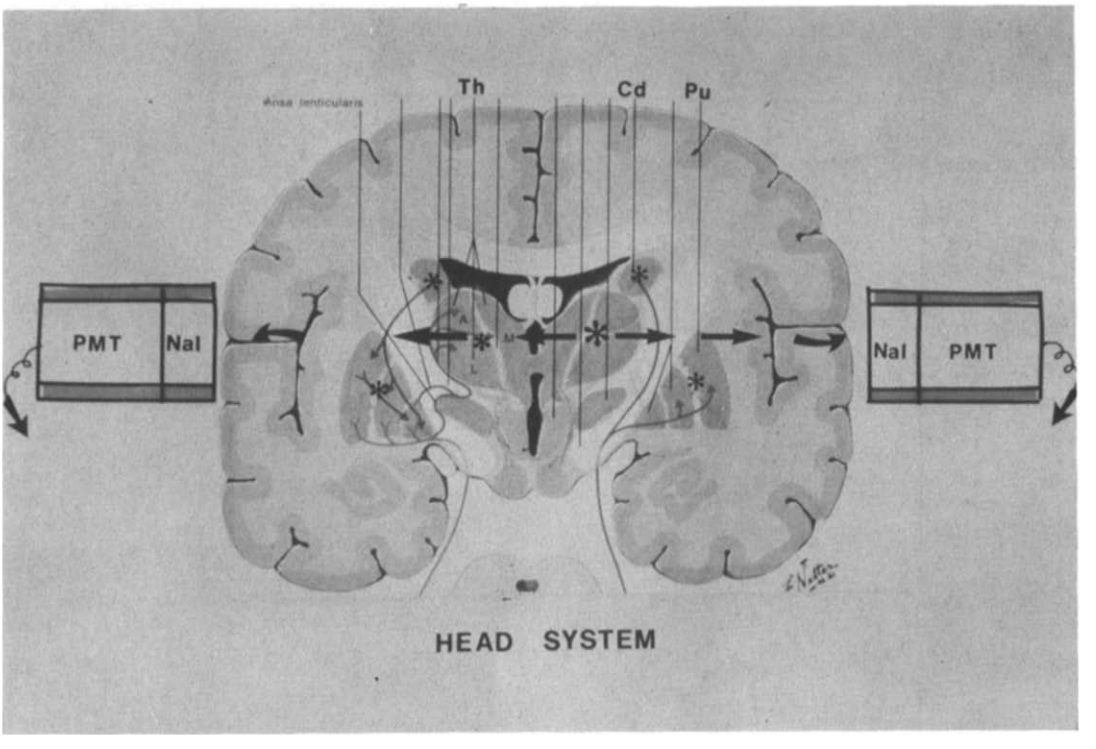

Fig. 6. A simple two detector system for assessing the rate of accumulation of positron-labelled drugs.

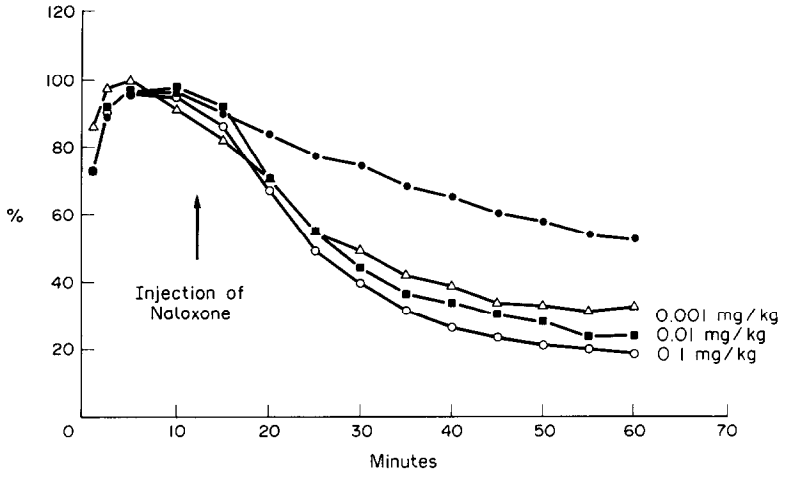

Fig. 7. Effect of naloxone in releasing carbon-11 carfentanil from opiate receptors as measured with the dual detector system in the human brain.

with bipolar depressive illness who have psychotic symptoms also have been observed to have elevated numbers of D2 dopamine receptors.

PARKINSON'S DISEASE

In Parkinson's disease, the number of D2 dopamine receptors were not reduced, a finding consistent with the fact that most patients with Parkinson's disease respond to the administration of bromocryptine or L-DOPA [23]. It would be of interest to determine the state of D2 dopamine receptors in patients with Parkinson's disease who are refractory to such drugs.

HOW IT BEGAN

Although the cyclotron was invented in 1931 by Nobel Laureate Ernest Lawrence, only recently have

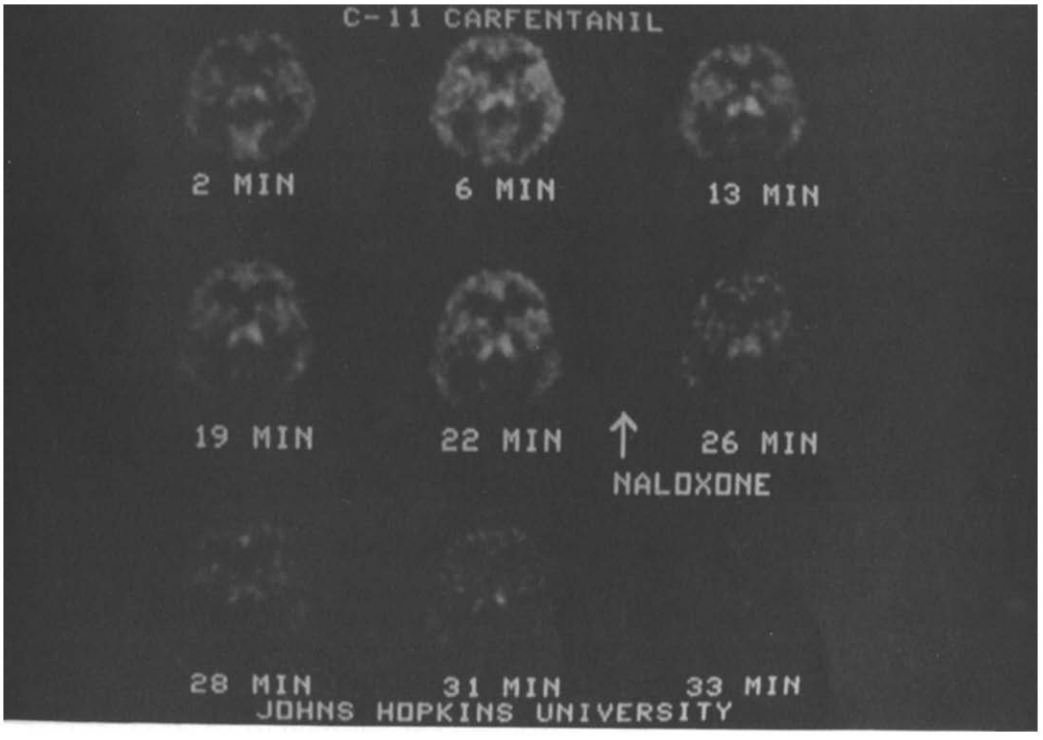

Fig. 8. Serial PET images illustrating the release of carbon-11 carfentanil by naloxone.

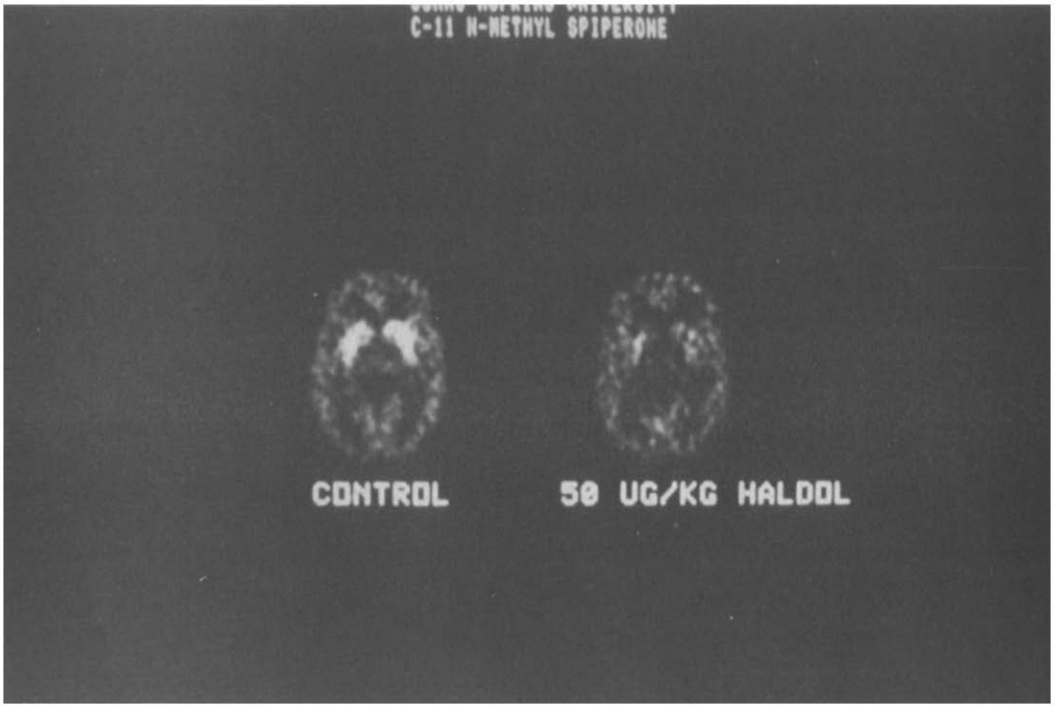

Fig. 9. Effect of prior ingestion of haloperidol on the accumulation of carbon-11 *N*-methyl spiperone by the caudate nucleus and putamen of a human being. The left image is the control image obtained 45 min after injection of the tracer.

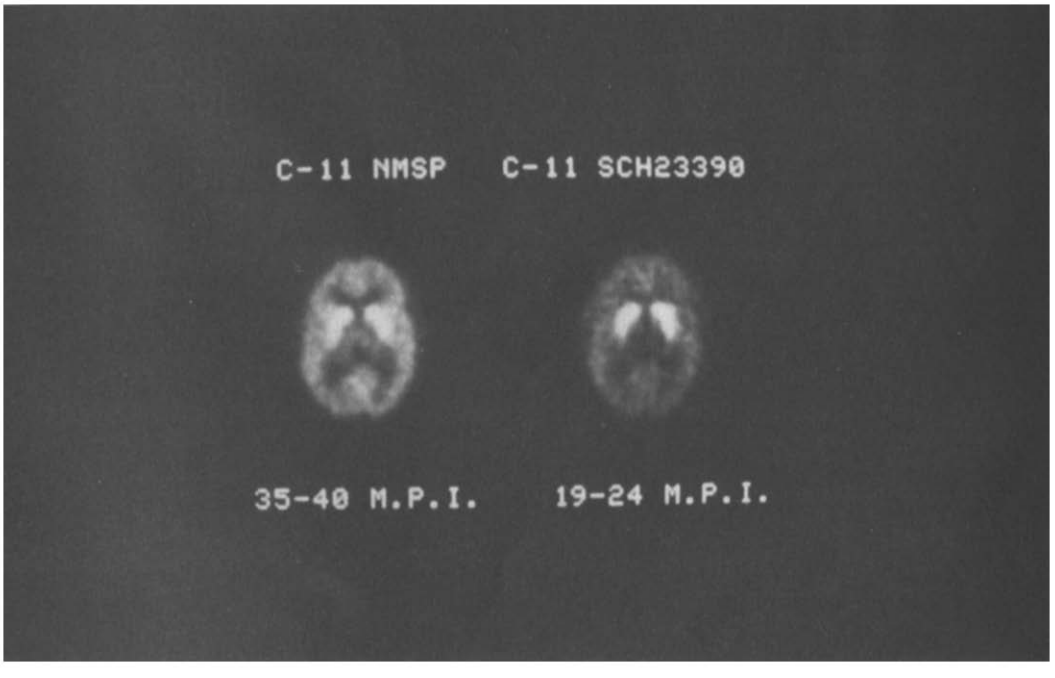

Fig. 10. Images of D1 (right) and D2 (left) dopamine receptors.

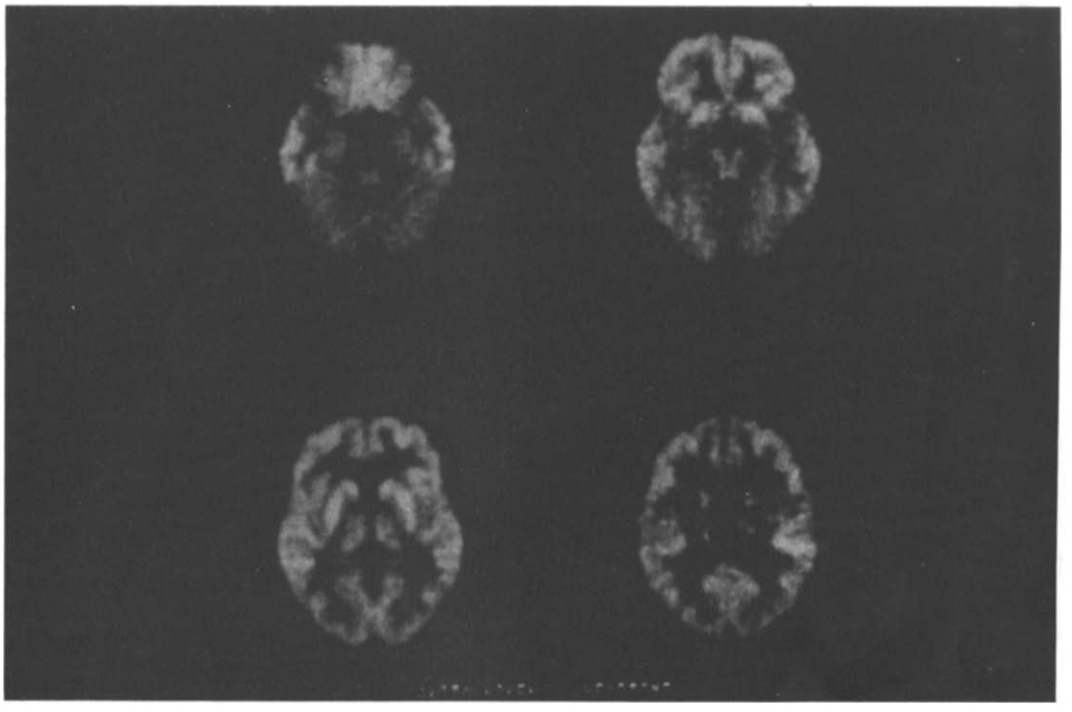

Fig. 11. Images of glucose metabolism at various levels of the brain of a normal person, imaged with a new Japanese PET scanner. Published with permission.

improvements in PET imaging and the simplification of the design and operation of cyclotrons brought about a renewed interest in positron-emitting radionuclides, such as carbon-11, fluorine-18, and oxygen-15. Over 40 years ago it was possible to demonstrate that there was decreased accumulation of radioodine by the thyroid in patients with hypothyroidism, and increased accumulation in hyperthyroidism. It has taken over 40 years for the tracer method to be extended to practically every organ of the body. In principle, every time we measure the rate of a chemical process *in vivo*, we raise the possibility of identifying at least two diseases, one in which the process is taking place at an unusually slow rate and another where the process is taking place at an abnormally fast rate.

When Fermi and his colleagues invented the nuclear reactor in 1941, and carbon-14 and tritium became available from the Manhattan District Project in 1946, cyclotrons were put on a back burner. These two radiotracers were cheaper and more readily available and revolutionized biochemistry. For *in vivo* studies, reactor-produced tracers, such as those labeled with technetium-99m, became the workhorses of the field of nuclear medicine. Inventors, such as Hal Anger, diverted their attention away from imaging instruments designed for positron-emitting radiotraces to those designed for radionuclides that emitted single photon/disintegrations, such as technetium-99m.

The combination of the Anger camera and technetium-99m led to procedures, such as brain scanning, to detect tumors and other mass lesion, but in 1977 the introduction of computed tomography resulted in an almost complete cessation of brain scanning with technitium-99m, and attention returned to the cyclotron as a source of radioactive tracers, because of its ability to produce biologically-important radionuclides, such as carbon-11 and fluorine-18. In the 10 year period between 1976 and 1986, the number of medical PET-cyclotron facilities in the world grew from 4 to 60.

REFERENCES

1. Phelps ME and Mazziotta JC, PET: Human brain function and biochemistry. *Science* **228**: 799–809, 1985.
2. Wagner HN Jr, Quantitative imaging of neuroreceptors in the living human brain. *Semin Nucl Med* **16**: 51–62, 1986.
3. Frost JJ, Studies of neurotransmitters and neuroreceptors by emission computed tomography. *Trends Pharmac Sci* 490–496, 1986.
4. MacGregor RR, Halldin C, Fowler JS, Wolf AF, Arnett CD, Langstrom B and Alexoff D, Selective irreversible *in vivo* binding of [^{11}C]clorgyline and [^{11}C]-L-deprenyl in mice: potential for measurement of functional monoamine oxidase activity in brain using positron emission tomography. *Biochem Pharmacol* **34**: 3207–3210, 1985.
5. Wong DF, Wagner HN Jr, Dannals RF, Links JM, Frost JJ, Ravert HT, Wilson AA, Rosenbaum AE, Gjedde A, Douglass KH, Petronis JD, Folstein MF, Toung JKT, Burns DH and Kuhar MJ, Effects of age on dopamine and serotonin receptors measured by positron tomography in the living human brain. *Science* **226**: 1393–1396, 1984.
6. Wagner HN Jr, Burns HD, Dannals RF, Wong DF, Langstrom B, Dueffler T, Frost JJ, Ravert HT, Links JM, Rosenbloom SB, Lukas SE, Kramer AV and Kuhar MJ, Imaging dopamine receptors in the human brain by positron tomography *Science* **221**: 1264–1266, 1983.

7. Dannals RF, Ravert HT, Frost JJ, Wilson AA, Burns HD and Wagner HN Jr, Radiosynthesis of an opiate receptor binding radiotracer: (^{11}C) carfentanil. *Int J Appl Radiat Isot* **36**: 303–306, 1985.
8. Frost JJ, Wagner HN Jr, Dannals RF, Ravert HT, Links JM, Wilson AA, Burns DH, Wong DF, McPherson RW, Rosenbaum AE, Kuhar MJ and Snyder SH, Imaging opiate receptors in the human brain by positron tomography. *J Comput Assist Tomog* **9**(2): 231–236, 1985.
9. Frost JJ, Behal R, Mayberg HS, Dannals RF, Ravert HT, Wilson AA, Links JM and Wagner HN Jr, Effects of naloxone on opiate receptor binding man measured by positron emission tomography. *J Nucl Med* **27**: P935, 1986.
10. Bice AN, Wagner HN Jr, Frost JJ, Natarajan TK, Lee MC, Wong DF, Dannals RF, Ravert HT, Wilson AA and Links JM, Simplified detection system for neuroreceptor studies in the human brain. J Nucl Med **27**: 184–191, 1986.
11. Garnett ES, Firnau G and Nahmias C, Dopamine visualized in the basal ganglia of living man. *Nature* **305**: 137–138, 1983.
12. Creese I, Bert DR and Snyder SH, Dopamine receptor binding predicts clinical and pharmacologic potencies of anti-schizophrenic drugs. *Science* **192**: 481–483, 1976.
13. Wong DF, Gjedde A and Wagner HN Jr, Quantification of neuroreceptors in the living human brain. I. Irreversible binding of ligands. *J Cerebral Blood Flow Metabolism* **6**: 137–146, 1986.
14. Wong DF, Gjedde A, Wagner HN Jr, Dannals RF, Douglass KH, Links JM and Kuhar MJ: Quantification of neuroreceptors in the living human brain. II. Inhibition studies of receptor density and affinity. *J Cerebral Blood Flow Metabolism* **6**: 147–153, 1986.
15. Muehlleher G and Karp JS, Positron emission tomography imaging—technical considerations. *Semin Nucl Med* **16**: 35–50, 1986.
16. Hirose Y, Kanno I, Yamamoto S, Amano M, Miura S, Iida H and Uemura K, A stationary sampling high resolution positron emission tomograph headtome IV: Design and preliminary data. *J Nucl Med* **28**: 607, 1987 (abstract 210).
17. London ER, Fanelli R, Szikszy M and Jasinski D, Effects of opioid analgesics on local cerebral glucose utilization. *NIDA Research Monograph 75, Progress in Opioid Research* (Eds Holladay J, Law P-Y and Henz A), pp 379–381, 1987.
18. Meyer MC, Straughton AB, Lo MW, Schary WL and Whiting CC, Bioequivalence dose proportionality, and pharmacokinetics of naltrexone after oral administration. *J Clin Psychiatry* **45** (9, section 2): 15–19, 1984.
19. Wall ME, Brine DR and Perez-Reyes M, Metabolism and disposition of naltrexone in man after oral and intravenous administration. *Drug Metab Dispos* **9**: 369–375, 1981.
20. Lee MC, Wagner HN Jr, Tanada S, Frost JJ, Bice AN and Dannals RF, Duration of occupancy of opiate receptors by naltrexone. Submitted for publication.
21. Verebey K, Volavka J and Mule SJ, Naltrexone: Disposition, metabolism and effects after acute and chronic dosing. *Clin Pharmacol Ther* **20**: 315–328, 1976.
22. Wong DF, Wagner HN Jr, Tune LE, Dannals RF, Pearlson GD, Links JM, Tamminga CA, Broussolle EP, Ravert HT, Wilson AA, Toung TK, Malat J, Williams JA, O'Tuama LA, Snyder SH, Kuhar MJ and Gjedde A, Positron emission tomography reveals elevated D2 dopamine receptors in drug-naive schizophrenics. *Science* **234**: 1558–1563, 1986.
23. Frost JJ, Uhl GE, Wong DF, Preziosi TJ, Dannals RF, Ravert HT and Wagner HN Jr, Dopamine receptor alterations in asymmetric and symmetric Parkinsonism measured by positron emission tomography. *Ann Neurol* **16**: 128A, 1984 (abstract).