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IN VIVO ASSESSMENT OF NEUROTRANSMITTER BIOCHEMISTRY IN HUMANS

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INTRODUCTION AND PERSPECTIVE

A biochemical understanding of human brain function in health and disease is a goal that has many dimensions. For the most part our knowledge of biochemical processes in the living human brain has come from inferences typically made from biochemical assays of body fluids or biopsy procedures, or has developed as an extension of findings in animal studies. In fact, only recently has a direct access to the biochemical nature of specific brain functions been possible in the living human being (1).

Investigations toward the elusive goal of assessing noninvasively the biochemistry of neuropsychiatric diseases are particularly challenging and of revolutionary significance. Until now, most of this knowledge has derived from the characterization of a variety of receptor systems using in vitro assay techniques (i.e. radioligand assays with membrane preparations, in vitro autoradiography) (2, 3) and, in a more limited way, in vivo autoradiographic characterization of receptor systems (4).

With the development of positron emission tomography (PET) (5), extension of these studies to humans became possible. Using this technique cholinergic (6), opiate (7, 8), benzodiazepine (9, 10), and dopamine receptor biochemistry (11, 12) has been studied in living animals and humans. The work carried out on the in vivo characterization of neurotransmitter systems has been mostly phenomenological, often directed to revealing receptor localization and number, the pharmacological character of ligand binding and

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the kinetic changes with physiological and pathophysiological alterations. Now we need to ask what this large collection of observations has taught us about in vivo neurotransmitter and receptor biochemistry. Because pre- and postsynaptic dopamine neurotransmission biochemistry has been the most extensively studied with PET, an attempt is made in this review to be critical in assessing progress in this area. This analysis is intended to serve as a model for other neurotransmitter systems less extensively studied in vivo with PET. A review on the clinical perspectives of neuroreceptor studies with PET has recently been presented (13).

POSITRON EMISSION TOMOGRAPHY: GENERAL PRINCIPLES

PET is an analytical imaging technique that permits the measurement of local, specific biochemical events in vivo (14). To obtain quantitative information of biochemical parameters with PET, three major components are required: (i) a positron tomograph; (ii) positron-emitting labeled tracers; and (iii) tracer kinetic mathematical models (1).

All radioisotopes used with PET decay by positron emission. A positron (positively charged electron) emitted from a decaying nucleus collides and then combines with an electron. The mass of the positron and electron annihilates into two 511 keV photons that are emitted at an angle of 180° from each other. These photons are detected by external detectors with coincidence circuits (5). *Positron tomographs* have a circumferential array of detectors for which data collected by annihilation coincidence detection are used to reconstruct mathematically the cross-sectional distribution of tissue radioactivity concentration into tomographic images.

This instrument is not only a tomographic imaging system, but a device that permits the estimation of quantitative biochemical or physiological rates in vivo, namely hemodynamic parameters, membrane transport, metabolic and biosynthesis rates (i.e. neurotransmitter synthesis fluxes), and receptor affinity and density. This is possible because biochemically or pharmacologically active compounds can be labeled with cyclotron-produced *positron emitting radioisotopes* of natural elements [i.e. ^{11}C (20.4 min half-life), ^{13}N (9.96 min), ^{15}O (2.03 min), ^{18}F (109.7 min)] (14).

Substitution of ^{11}C, ^{13}N, and ^{15}O for the natural isotopes of carbon, nitrogen, and oxygen, respectively, renders compounds biochemically indistinguishable from their natural counterparts. Fluorine-18 on the other hand is frequently used to provide labeled substrate analogs (e.g. 2-deoxy-2-[^{18}F]fluoro-D-glucose) or pharmacological agents (e.g. ^{18}F-labeled neuroleptic drugs) that trace biochemical processes in a predictable manner (14).

The third major component of PET, *tracer kinetic models,* provides a

framework for calculation of the rates of processes under study. At present many biologically active compounds are available for measuring biochemical or pharmacological variables. Several hundred positron labeled compounds have been synthesized to date. The list includes radiolabeled carbohydrates, free fatty acids, amino acids, and a variety of pharmacological agents. However, the availability of radiotracers is a necessary but insufficient condition for performing specific assay measurements. Analytical studies of biochemical processes with PET are meaningful only with radiolabeled compounds that can be accurately modeled in vivo using tracer kinetic principles (14, 15).

Tracer kinetic models are, therefore, a necessary and major component of PET. The positron tomograph only measures local time-dependent radiolabeled concentration changes throughout the organ (i.e. brain). For conversion of these images into local reaction rates, PET measurements must be combined with the time course of radiotracer in blood and integrated with validated tracer kinetic models of the process under study.

The spatial resolution of PET scanner designs ranges from about 2.5 to 4.5 mm (16), significantly less than the 1 to 2 mm resolution of proton magnetic resonance imaging (MRI). This apparent disadvantage in resolution, however, is compensated by another property of PET: its enormous sensitivity (14) which allows measurements of chemical reactions involving constituents whose concentrations are in the picomolar range. Therefore, PET (a true tracer technique) is as such ideally suited for the in vivo investigation of the dynamic of biochemical processes involving neuroreceptors, whose tissue concentrations are typically in the nanomolar range (17, 18).

PROBING NEUROTRANSMITTER SYSTEMS IN VIVO

The ability of PET to map the entire brain for local dynamic biochemical processes offers a powerful tool to identify neuronal sites and functional activity of neurotransmitter systems in the living human brain. Of all neurotransmitter systems, the dopamine system has been the most widely investigated in a growing number of PET centers around the world, probably because a variety of neuropsychiatric diseases (e.g. schizophrenia, Parkinson's disease, tardive dyskinesia, Huntington's disease) (13) and behavior modifying drugs have been associated with alterations in the dopaminergic system.

To date, the most extensive PET work with the dopaminergic system deals with postsynaptic D2 receptors, which can be easily imaged with radiolabeled ligands in vivo. Also presynaptic characterization of the dopaminergic system has been made in vivo with positron-emitting labeled probes, aided by the extensive biochemical data available on dopamine biosynthesis, reuptake,

metabolism, and enzymatic regulation. On the other hand, dopamine autoreceptors have not yet been studied in vivo with PET (Figure 1). The dopaminergic system is used in this review as a model system to exemplify the approaches used in PET to examine the chemical basis of neurotransmission in vivo in humans.

Dopamine Neuroreceptors

The term *receptor* has been coined to denote a macromolecule (or complex system of macromolecules) that serves the function of neurotransmitter recognition to mediate transmission of an impulse (2). For some dopaminergic responses, Greengard and coworkers (19) demonstrated that the presynaptically released dopamine, upon interaction with postsynaptic receptors, activates adenylate cyclase, an enzyme that catalyzes the conversion of ATP to cyclic-AMP. Cyclic AMP, the second messenger, initiates a cascade of events resulting in altered membrane permeability with concomitant modifications in neuronal activity. To confirm this observation it was demonstrated that the anatomical localization of the dopamine-sensitive adenylate cyclase in brain tissue was also similar to that of dopamine receptors, namely in corpus striatum, olfactory tubercle and nucleus accumbens (17).

Many antipsychotic or neuroleptic drugs used in the treatment of schizophrenia are known dopamine antagonists. Little correlation, however, was found between their pharmacological activity in vivo and their biochemical

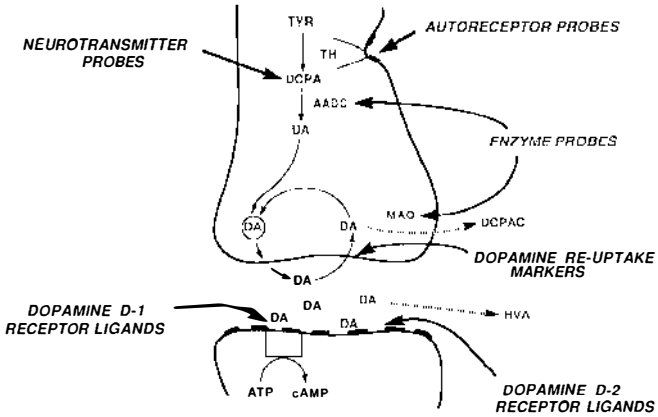

Figure 1 Schematic illustrations of the central dopaminergic nerve synapse with pre- and postsynaptic elements. Presynaptically the synthesis, regulation, metabolism, and reuptake of dopamine (DA) is depicted. Postsynaptically, Dl (adenylate cyclase linked) and D2 receptors are shown. Also the site of action of labeled pre- and postsynaptic probes for PET studies is indicated. TYR: tyrosine; DOPA: 3,4-dihydroxyphenylalanine; DA: dopamine; HVA: homovanillic acid; DOPAC: 3,4-dihydroxyphenylacetic acid; TH: tyrosine hydroxylase; AADC: aromatic amino acid decarboxylase; MAO: monoamineoxidase (mainly intraneuronal); COMT: catechol-0-methyltransferase; ATP: adenosine triphosphate; cAMP:cyclic AMP.

potencies on adenylate cyclase (17). This discrepancy raised the possibility that neuroleptics (e.g. butyrophenones) may act by binding at a different subpopulation of dopamine receptors (20).

Following considerable controversy (for review see 21), it is now generally accepted that there are two different subpopulations of dopamine postsynaptic receptors, namely: D1, linked to the stimulation of the activity by adenylate cyclase, and D2, with negative association with this enzyme. Presynaptic dopamine receptors, or autoreceptors, have also been demonstrated in the brain (21, 22). These autoreceptors appear to modulate the rate of dopamine biosynthesis by a negative feedback mechanism. According to this hypothesis, dopamine released into the synaptic cleft binds to the autoreceptor with high affinity (nM range), modifying tyrosine hydroxylase (TH) activity and inhibiting dopamine synthesis (22, 23). Presynaptic dopamine receptors may play an important physiological role in the modulation of dopamine synthesis and release (22, 23). By responding to the amount of dopamine in the synaptic cleft, synthesis is depressed with high concentrations of dopamine and increased when dopamine concentration is low.

The mechanism of inhibition of TH is in fact more complicated than the process of end-product inhibition. The kinetic characteristics of the enzyme are also affected by depolarization of catecholamine terminals, reversible phosphorylation mechanisms, (24) and by the presence of multiple neurotransmitters (25).

DOPAMINE D2 NEURORECEPTOR ASSAYS Review of the literature reveals that in vivo neuroreceptor binding assays with PET depend critically on the selection of the radiolabeled tracer. Radiotracers for in vivo receptor assay with PET should, in fact, meet specific criteria (13, 18). They should have (*a*) receptor specificity, selectivity, and saturability (in vivo and in vitro), (*b*) low degree of nonspecific binding, (*c*) metabolic stability in vivo, and (*d*) high-specific activity. Obviously, for successful brain studies, brain permeability of the radiotracers is assumed.

Receptor specificity, selectivity and saturability In vivo dopamine receptor binding assays in humans were initially stimulated by in vivo studies in rodents with [^{3}H]spiperone, a neuroleptic ligand with high affinity for dopamine D2 receptors ($K_D < 10^{-9}$ M) (26, 27). Even though spiperone also binds in vivo to serotonin receptors (28), these studies demonstrated that [^{3}H]spiperone binding is regionally selective in the brain, with anatomic differentiation in the brain structures containing dopamine and serotonin receptors. Several hours after intravenous administration of [^{3}H]spiperone, the radiotracer accumulated in areas with high dopamine (corpus striatum, nucleus acumbens, and olfactory tubercles) and serotonin (frontal cortex) receptor densities, and it was rapidly washed out from cerebellum, an area

believed to lack dopamine receptors (28). The high striatum: cerebellum ratios (10 at 3 h) observed after administration of [^{3}H]spiperone were immediately suggestive that labeling of dopamine D2 (and serotonin S2) receptors in the living human being was possible.

Friedman and coworkers (29) first demonstrated (using [^{77}Br]-spiperone and single photon emission computed tomography) the possibility of visualizing areas with high dopamine-receptor density in cats. Subsequently, Wagner and coworkers (12), using 3-[^{11}C]methylspiperone (^{11}C NMSP), extended this work to humans. Such experiments were followed by others with various positron-emitting labeled neuroleptics (30–32). In all cases, when suitable radioligands are used, binding kinetics obtained with serial PET scans in nonhuman primates and humans have features similar to those observed in rodents with [^{3}H]spiperone (Figure 2).

Preliminary evidence that in vivo radiolabeled neuroleptic binding involves specific receptors was inferred from regional localization of radioactivity in appropriate neuroanatomical systems. A useful determinant of binding specificity is the isomeric *stereospecificity*, shown by (+)butaclamol that blocks in vivo neuroleptic binding (33). High affinity competition binding of a ligand with (+)butaclamol has been considered to demonstrate binding to neuroleptic sites, either dopaminergic or serotonergic (34). Two other requisites are required to complete the criteria for receptor identification, namely: (*a*) binding *saturation* with increasing concentrations of radioligand receptor (17), and (*b*) *biochemical analyses* in animals (frequently rodents) revealing that the specifically bound striatum activity is the authentic radioligand injected (17).

Discrepancies have been observed between in vitro and in vivo radiolabeled neuroleptic receptor binding. The most notorious of these discrepancies relates to the slow clearance from brain structures in vivo (e.g. >16 hr for

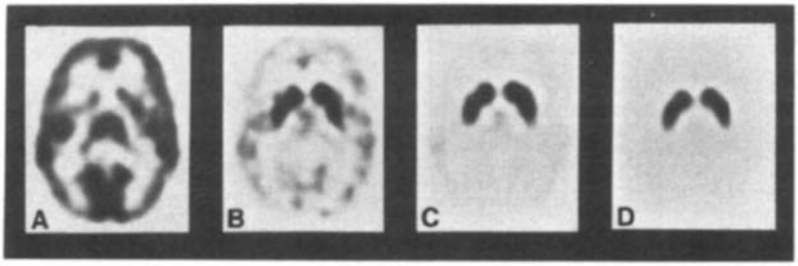

Figure 2 A typical set of human brain PET images of FESP at various times after intravenous bolus injection of the tracer. The tracer is delivered to tissue by blood flow, as shown by the resemblance of the early image (Set A, 90 sec) to cerebral blood flow distribution. Gradually, the tracer is selectively accumulated in the caudate and putamen that are rich in dopamine D2 receptors (Set B, 60 min). The caudate to cerebellum ratio of radioactivity is about 3.3 at 110 min. (Set C) and 11 at 420 min. (Set D).

spiperone) (35), when compared with that observed in vitro (10 min for spiperone) (26). The in vivo clearance of spiperone is longer than expcctcd from its dissociation constant measured under in vitro conditions. A further paradox between in vitro and in vivo conditions exists because in the former spiperone binding is competitive with dopamine and dopamine agonists, whereas in the latter spiperone binding is mediated by these compounds. It has been recently postulated that the slow striatal clearance of spiperone can be explained by the mechanism of postsynaptic dopamine-mediated receptor internalization and recycling (36). This postulate is also consistent with the incomplete in vivo post-binding displacement of radiolabeled butyrophenones that occurs upon administration of structurally related nonradioactive butyrophenones (35, 37).

Nonspecific binding Among the simplest principles of in vivo neuroreceptor assays with PET stands the need to have a high, time-dependent, specific anatomical localization of the radiotracer with minimum nonspecific binding. This is required to allow clear delineation of specific binding over a background of nonspecific distribution. The necessity of this requirement stems from the fact that PET separates specific and nonspecific binding by differences in the temporal course (kinetics) of local tissue concentrations. It can be stated unequivocally that successful quantitation of radiolabeled ligand binding to dopamine receptors in the brain requires not only an understanding of the nature of receptor-ligand interactions, but also the nonspecific binding characteristics of the radiotracer used. For example, pimozide, a neuroleptic butyrophenone with an in vitro binding affinity for dopamine D2 receptor similar to that of spiperone (17), presents a low in vivo target (striatum)/nontarget(cerebellum) binding ratio. Its higher hydrophobicity, when compared with spiperone, produces a slow clearance from nonspecific sites and poor kinetic and tissue concentration separations from specific sites (38). Similar observations with [^{18}F]haloperidol forbid its effective use in vivo (39).

Seeman in his comprehensive review (33) indicates that the high hydrophobicity of neuroleptic drugs makes them surface active and very soluble in biological membranes. One could expect, therefore, that nonspecific binding, believed to be primarily the result of hydrophobic interactions, ionic attractions, and van der Wall forces (17, 33), may not be displaceable by drugs that interact with the dopamine D2 receptor. Obviously nonspecific binding interactions, with lipids or proteins in biological membranes or intracellular compartments, are weaker (lower binding affinity) than specific ligand-receptor interactions. However, nonspecific binding sites vastly outnumber the specific receptor sites and thus can still account for a significant fraction of total radiotracer binding (17).

An added complication for quantitative receptor binding analyses is that,

according to Seeman (33), two types of nonspecific binding were identified: (*a*) a nonspecific, nonsaturable component observable in the presence of an excess concentration of the same nonradioactive compound, and (*b*) a nonspecific, saturable site determined in the presence of an excess concentration of the closest congener [e.g. (+)butaclamol] of the radiotracer. Ideally, these two nonspecific components should be identical, but they rarely are, even for radiolabeled spiperone (40).

Metabolic stability One of the necessary conditions for quantitative estimation of receptor density and affinity relates to the metabolic stability of the radiotracer used in the in vivo studies. Two factors are decisive to determine the usefulness of radiolabeled receptor ligands. (*a*) No significant brain metabolism of the radiotracer occurs during the experimental period. (*b*) Even though most drugs, including butyrophenone neuroleptics, are rapidly metabolized peripherally, it is expected that plasma metabolites of the radiolabeled tracer are not transported into the brain. If labeled metabolites do enter the brain they should not compete for receptor sites. Spiperone is not metabolized in brain but is, indeed, rapidly metabolized peripherally (41). However, none of the plasma metabolites appear in rat striatum up to 2 hr after injection (41), at times when only 15% of the plasma activity (when spiperone labeled with ^{3}H is used) is associated with spiperone. Oxidative N-dealkylation and subsequent biodegradation of the butyrophenone chain constitute the major metabolic pathway for butyrophenones. When tritiated butyrophenone neuroleptics have been used in rodents, 3-(4-fluorobenzoyl)propionic acid, 4-fluorophenylacetic acid, and 4-fluorophenylaceturic acid have been observed as metabolic products (42, 43). The nonpermeability of 3-(4-[^{18}F]fluorobenzoyl) propionic acid across the blood brain barrier suggests that these acidic metabolites, formed in the periphery, do not enter the brain (44).

Equally limited is the information available about the piperidine metabolites arising from oxidative N-dealkylation of butyrophenones. In vitro studies (45) have identified 4-(p-chlorophenyl)-4-hydroxypiperidine and 4-hydroxy-4-(α,α,α- trifluoro-m-tolyl)piperidine as metabolites of N-dealkylation of haloperidol and trifluoroperidol, respectively. Analogous piperidine metabolites were identified by biotransformation of other neuroleptics (see, for example, 46). Also in the case of fluspirilene, a triazaspirodecanone neuroleptic related to spiperone, the formation of a spirohydantoin metabolite has been recognized (47).

Only limited information is available as to the permeability of these piperidine-containing metabolites into the brain. However, studies with radiolabeled neuroleptics that could potentially produce radioactive metabolites containing the triazaspirodecanone moiety—i.e. 3-[^{11}C]methylspiperone (12), 3-(2'-[^{18}F]fluoroethyl)-spiperone (FESP) (32)—indicate that the overall

effect, if any, of radiolabeled metabolites delivered to the brain by plasma is small (37, 48). For example, biochemical analyses of rat brain after intravenous injection of FESP revealed that, up to four hours, 90% of striatum activity is the authentic radiotracer (37, 48).

Independent of this consideration, and even though receptor imaging and quantitation may not necessarily be affected by peripheral metabolism, appropriate chemical characterization of arterial input functions are required for quantitative estimates of receptor number and affinity. In all cases, therefore, corrections for metabolism must be performed. This requirement was not uniformly observed in early work until its significance was demonstrated (41).

Specific-activity of radiolabeled receptor ligands Receptor concentrations in the central nervous system are low (1–25 pmole/g tissue) (17, 18), making the need of high-specific activity (200–10,000 Ci/mmol) radiolabeled ligands for in vivo studies with PET immediately apparent. This is not a trivial requirement if one considers that [^{3}H]haloperidol with a marginally useful specific activity for receptor studies (10.5 Ci/mmole) only became available in 1974 (33). Before any human study was performed, Frost (18) actually estimated the minimum useful specific activity for [^{18}F]spiperone labelling of striatal dopamine receptors in vivo in humans to be 11.2 Ci/mmol. Lower specific activities, for a given amount of radiolabel injected, also imply high masses of the tracer, which has the effect of (*a*) increasing the level of nonspecific binding, and (*b*) producing significant receptor occupancy with the consequent pharmacological activity. Actually, a 10 mCi dose of [^{18}F]spiperone, well within the limits required by human dosimetry (39), with a minimum specific activity of 11.2 Ci/mmol, represents a dose of 5 μg/Kg, very close to the therapeutic dose of spiperone (6 μg/Kg/day) (49).

After earlier, rather unsuccessful attempts with [^{11}C]chlorpromazine (50) and [^{11}C]fluonitrazepam (9) to visualize central receptor binding, butyrophenone neuroleptics became targets in the search for high specific activity, high affinity radioligands to label the dopamine receptor in vivo (14). Butyrophenone neuroleptics were labeled with short-lived radionuclides such as ^{11}C (51, 52), ^{18}F (32, 53–56) ^{75}Br, ^{76}Br and ^{77}Br (29, 57, 58). Fluorine-18 labeled radiotracers offer the advantage that they permit tomographic evaluations of binding kinetics for many hours, and they facilitate the estimation of various kinetic parameters (Figure 2). On the other hand, a potential advantage of using ^{11}C is that serial studies in a single setting can be carried out by virtue of its shorter half-life (30).

Available positron-emitting bromine radionuclides have longer half-lives (^{75}Br: 1.5 h; ^{76}Br: 16.2 h) than does ^{11}C and are, therefore, more convenient for chemical incorporation into organic molecules. They suffer from two

drawbacks, however: (*a*) Their preparation requires large cyclotrons since they are typically produced using the (^{3}He, 3n) reactions on arsenic targets. (*b*) Their emission characteristics are not ideally suited for tomographic studies (59).

These considerations help us to understand the considerable initial interest in the incorporation of ^{18}F at the para position on the phenyl ring of the butyrophenone moiety in spiperone and haloperidol (60). However, despite intensive efforts, all these synthetic methods either give poor radiochemical yields and have low reproducibility (54, 60) or involve labor intensive, time consuming, multistep reactions (55, 56). It was the observation that 3-N-alkylation of spiperone (12) and its brominated analogs (58) does not drastically affect binding to the dopamine receptor that prompted the most recent successful synthesis and tomographic evaluation of 3-N-fluoroalkylspiperone analogs (32, 48, 53).

Quantitative estimates The use of high affinity ligands (e.g. most radiolabeled neuroleptics) can provide high specific to nonspecific localization in vivo with PET (Figure 2). This satisfies one of the initial criteria for performing quantitative estimates of receptor density (for review see 61). One type of tracer kinetic modelling approach uses the dynamics of the tracer uptake and clearance in brain tissues and in plasma after radiotracer injection. For labeled ligands of high binding affinity and high specific activity, (e.g. spiperone), the dynamic approach gives the product of the association constant (ka) and receptor density (Bmax), referred to as "the binding potential" (62). The use of either a lower specific activity injection of the labeled ligand (i.e. with a larger amount of nonradioactive tracer) or a mass amount of a competitive ligand to saturate partially the neuroreceptors is required to decouple the estimated value of Bmax from that of ka. The latter approach has been used by Wong et al with C-11 NMSP to measure dopamine D2 receptor densities in the caudate of normal human subjects (63), as well as drug-naive and drug-treated schizophrenics (64).

Nevertheless, a relatively high receptor occupancy with consequent pharmacological effects may be unacceptable in most cases, particularly with patients with compromised dopamine neurotransmission (e.g. Parkinson's disease). The magnitude of this problem has been much reduced with the use of lower affinity ($K_D > 10^{-9}$ M) substituted benzamides (e.g. [^{11}C]raclopride) (65). The neurochemical spectrum of these benzamides generally resembles that of other neuroleptic drugs in that these compounds produce blockade of dopamine receptors (66) but are generally devoid of extrapyramidal effects. Carbon-11 raclopride has been used in PET studies with doses that are in excess of 6.7 μg/Kg without observed pharmacological effects (13). Radiolabeled benzamides have, therefore, the advantage over butyrophenone neuroleptics of a reduced pharmacological risk during PET

studies with humans. They achieve also a rapid equilibrium in vivo in the brain enabling the estimation of receptor densities and affinity (13, 61) with an approach that is equivalent to the in vitro equilibrium receptor binding assays (2). However, PET studies with [^{11}C]raclopride have been performed only for up to 60 min (13) and, even though the extensive metabolism of the antipsychotic benzamides is known, (67) the possibility of metabolism of [^{11}C]raclopride during PET studies has not been addressed yet.

DOPAMINE D1 NEURORECEPTOR ASSAYS The lack of specific receptor ligands and the relatively meager biochemical and pharmacological information available on dopamine D1 receptor subtypes have contributed to a slow progress in this area. It was only recently that the benzazepine derivative R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5,-tetrahydro-1H-3-benzazepine (SCH 23390) was characterized as a highly selective D1 dopamine receptor antagonist (68, 69). This observation led to the preparation of ^{11}C-labeled SCH 23390 (70), as well as ^{75}Br, ^{76}Br labeled (71) analogs of this compound. Preliminary PET studies with these derivatives suggest that SCH 23390 may be a promising ligand for the characterization of dopamine D1 receptors in vivo (70).

Assessment of the Functional Integrity of the Presynaptic Dopamine System

Tyrosine is the primary substrate for catecholamine synthesis in nerve terminals (Figure 1). The transformation of tyrosine to L-dopa (3,4-dihydroxyphenylalanine) (72) is catalyzed by tyrosine hydroxylase (TH), a mixed function oxidase that utilizes molecular O_2 and biopterin as a cofactor (24). TH has a Michaelis constant (Km) for tyrosine in the micromolar range. Although TH is substrate-saturated under normal physiological conditions, it is kinetically regulated by a variety of mechanisms (see section on Dopamine Neuroreceptors).

Aromatic amino acid decarboxylase (AADC) catalyzes the conversion of L-dopa to dopamine in the cytosol (24). The synthesized dopamine is subsequently stored within vesicles in an ATP-dependent mechanism (73). When a stimulus reaches the nerve terminal, the vesicles, in a process mediated by Ca^{2+}, discharge their catecholamine content in the neuronal cleft. Dopamine binding to the postsynaptic receptor activates postsynaptic mechanisms for transmission of the action potential originating presynaptically. This action is primarily terminated by a carrier mediated, energy-dependent presynaptic reuptake mechanism (74), with subsequent vesicular storage of dopamine. Excess of free intracellular dopamine is inactivated by mitochondrial monoamineoxidase (MAO), whereas extraneuronal dopamine is inactivated by the Mg^{2+}-dependent catechol-0-methyl transferase (COMT) (24).

The functional integrity of the presynaptic dopaminergic mechanisms in the central nervous system is now being investigated with PET. For example, C-11 labeled nomifensine has been recently used to evaluate dopaminergic reuptake mechanisms at presynaptic terminals with tomographic experiments in monkeys (75). It is, however, the in vivo assessment of dopamine synthesis fluxes in nerve terminals with 6-[^{18}F]fluorodopa (FD) that has received most of the attention. FD is reversibly transported across the blood brain barrier. Its transport into brain is inhibited by infusion of neutral amino acids, which is consistent with the notion that FD is competitively transported by the neutral amino acid transport system at the blood brain barrier (76). In brain tissue, FD is decarboxylated by AADC to 6-fluorodopamine (FDA) and subsequently metabolized. In fact, FD acts as a competitive substrate with L-dopa to yield fluorinated products, namely 3-0-methyl-6-fluorodopa (3-OMFD), FDA, 3,4-dihydroxy-6-fluorophenylacetic acid (FDOPAC) and 6-fluorohomovanillic acid (FHVA) (77).

Chiueh et al were also able to show that after intraventricular injection of FD in rats there was potassium stimulated release of FDA, indicating that FDA can serve as a false transmitter in dopaminergic terminals (78). Further evidence is provided, as indicated above, by the presence of metabolites such as FDOPAC and FHVA in the striatum of rats given FD. Garnett et al pioneered the study of FD in primates (79) and also found that accumulation of radioactivity in the striatum is related to the formation and storage of FDA within intraneuronal vesicles. The striatal activity can be discharged following administration of reserpine, a compound known to deplete dopamine stores.

Initial work to evaluate pre- and postsynaptic striatal dopamine neurotransmission in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treated monkeys with moderate to severe Parkinsonian symptoms has also been undertaken (80). After administration, MPTP is capable of producing a highly selective degeneration of the zona compacta of the substantia nigra in various animal species (81). The compound produces an irreversible Parkinsonian syndrome in humans and when used in monkeys has provided a primate model for human Parkinson's disease. Histopathological changes noted in primates exposed to an MPTP regimen show characteristic neuronal cell loss, astrocytosis, and extracellular melanin in the substantia nigra compacta. The biochemical changes that occur with the administration of MPTP are immense, particularly in drastic reduction of dopamine content (>80%), compared with normal values. It has also been documented that tyrosine hydroxylase activity and aromatic amino acid decarboxylase activity are markedly reduced in the striatum of animals exposed to MPTP. In FD-PET scans of monkeys with severe MPTP-induced Parkinsonian symptoms, no visualization of the caudate occurs, as one would expect with profound presynaptic cell

losses. On the other hand, when FESP and PET were used, no significant difference in the estimated Bmax (24.3 ± 12.0 pmole/g) was found between the MPTP-treated (symptomatic) and the normal control animals. Consistent with this observation in vivo, in vitro [^{3}H]spiperone binding in these animals showed a receptor (dopamine D2) density (Bmax) of 21.2 pmol/g tissue. Also, histopathologic and immunohistochemical analysis showed an almost complete loss of dopaminergic cell bodies in the nigra and severely reduced tyrosine hydroxylase activity in the caudate. These results are indicative of the usefulness of FD for selectively assessing presynaptic neuronal degeneration in vivo.

The first demonstration of the regional distribution of FD in the brain of a normal man was obtained in 1983 by Garnett and colleagues (82). To date, the presynaptic dopaminergic pathway has been studied with PET and FD in normal individuals, MPTP exposed patients, and in patients with Parkinson's disease. Patients with hemi-Parkinsonism reportedly have reduced activity in the putamen on the contralateral side of the motor deficit, but normal caudate activity (83). Patients with bilateral Parkinson's disease, have a mild symmetric decrease in caudate activity accompanied by more severe reductions in the putamen (84), consistent with preexisting neurochemical data showing that dopamine depletion is more marked in the putamen than in the caudate in Parkinson's disease. With MPTP-induced human Parkinsonism (85), a striatal decrease in FD accumulation was noted, indistinguishable from results obtained in patients with Parkinson's disease.

From a methodological perspective, it should be stated that interpretation of FD-PET studies is complicated by a significant background activity in all brain structures consisting of unmetabolized FD and 3-OMFD formed by catechol-0-methyl transferase (COMT) in brain and peripheral tissues. One of the major difficulties in structuring a quantitative assay model for interpretation of the kinetics measured with PET after injection of FD can be traced to the complexities of FD membrane transport and metabolism, particularly the peripheral and cerebral formation of 3-OMFD. Therefore, other procedures have been proposed to assess the functional integrity of the presynaptic dopaminergic systems with PET, most notably the assessment of AADC activity in the human brain using radiotracers that would label the enzyme irreversibly, in proportion to its enzymatic activity (14). The parallel distribution of AADC activity and that of the corresponding neuroamines (e.g. dopamine in corpus striatum) is taken to indicate that the enzyme resides intraneuronally and, therefore, can be used to estimate the densities of dopamine neurons (86). This possibility is of considerable practical significance because it would permit a quantitative estimation of dopaminergic cell losses in degenerative extrapyramidal disorders (e.g. in Parkinson's disease or MPTP-treated primates).

CONCLUSIONS

Using positron emission tomography, in vivo pharmacological data can be obtained from normal human subjects and patients with cerebral disorders. Drugs (i.e. spiperone and derivatives, raclopride) can be labeled with positron-emitters and their pharmacokinetic behavior examined in vivo under tracer conditions or even at concentrations producing pharmacological effects. Following these procedures, the effect of specific pharmacological agents on behavior or symptoms can be observed and correlated with alterations in pharmacokinetics at their sites of action in the brain (13). These studies are providing for the first time the opportunity to examine the chemical dynamics of neurotransmissions in the brain of the living human, as well as the means for assessing the biochemical basis of neuropsychiatric diseases.

Significant efforts are required to structure and refine the analytical assay methods using existing or newly proposed radiotracers. The biochemical meaning and significance of parameters estimated with these assay methods must be documented, as well as their relationship to changes in the ultimate cerebral function, namely behavior. This requires the development and appropriate use of tracer kinetic models to document the time course of radiotracer transport and metabolism as well as thorough biochemical characterization of the radiotracers. Once these methods are validated, it must be shown that changes in model parameters occur when changes in behavior are elicited.

In the dopaminergic system, PET is now being used to investigate alterations of receptor density in humans as a result of chronic neuroleptic treatment, schizophrenia, and affective disorders. Studies are being performed to examine the relationship between the number of blocked and exposed receptors as a function of neuroleptic dose, plasma concentration of neuroleptics, and behavioral changes in schizophrenics. Presynaptic (e.g. Parkinson's disease) and post synaptic (e.g. Huntington's chorea) degeneration are now being studied with PET. Assays for enzymes along the synthesis and regulatory pathways of the dopaminergic system are also being developed to allow isolation of specific deficits in this system. The number and accuracy of these types of assays are continually increasing. This allows a unique access to the examination of the neurochemical basis of normal and abnormal behavior, as well as the effect of pharmacological manipulations in the brain of living subjects.

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