CAMBRIDGE

Cambridge University Press
978-0-521-79002-4 - Imaging Dopamine
Paul Cumming
Excerpt
More information

Introduction

This book is a biography of dopamine, as illuminated by classical neurochemical methods and especially by molecular imaging with positron emission tomography (PET), or single photon emission tomography (SPET), a closely related technology.

Since the early 1980s, molecular imaging has become indispensable for the study of normal physiology, disease processes, and novel therapeutics. Using external detection with PET or SPET, the uptake and metabolism or binding of radioligands is monitored and quantified in living brain. This book summarizes the state of knowledge of the half-dozen molecular targets in the dopamine system, which have been investigated by imaging techniques. A key advantage of molecular imaging is that aspects of the life of dopamine can be studied in living brain, both in preclinical studies and in humans afflicted with neurodegenerative or psychiatric disorders in which dopamine is implicated. A key disadvantage is presented by the type of knowledge obtained by molecular imaging, which can be only indirectly informative of the step of the pathway for dopamine neurotransmission under investigation. Thus, the interpretation of molecular imaging results must always be grounded in basic aspects of the biochemistry of dopamine and the pharmacology of its binding sites.

Although several groups of dopamine neurons are found in the brain, the entire emphasis here is to be placed on the mesencephalic dopamine systems, which innervate the extended striatum and specific limbic structures of the forebrain. In the course of a long human life, perhaps 10 g of dopamine is formed in the striatum, to be gradually released in infinitesimal amounts from individual dopamine nerve terminals. Once released from synaptic vesicles to the extracellular milieu, dopamine binds to its receptors and so conveys the dopamine signal to receptive neurons. While the concentration of dopamine in synaptic

© Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
978-0-521-79002-4 - Imaging Dopamine
Paul Cumming
Excerpt
More information

vesicles is very high, the prevailing concentration of dopamine in the extracellular space, also known as the interstitial compartment, is very low, comparable to the sweetness obtained by dissolving a sugar cube in a large swimming pool. Nonetheless, dopamine at these trace concentrations plays a substantial role in brain signaling and has been implicated in diverse neurological and psychiatric disorders.

The advent of the modern era of dopamine research was announced when Arvid Carlsson showed that the *Rauwolfia* alkaloid reserpine depleted the concentration of dopamine in the brain and other tissues of rabbits. In the condition of dopamine depletion, the rabbits were incapacitated by rigid paralysis, but restoration of brain dopamine and also normal movement was obtained after treatment of the sick rabbits with the dopamine precursor, the amino acid 3,4-dihydroxy-L-phenylalanine (levodopa; DOPA) (Carlsson, Lindqvist, & Magnusson 1957). This fundamental observation ultimately led to the awarding of the Nobel Prize in Medicine (2000), shared by Arvid Carlsson, Paul Greengard, and Eric Kandell. The progress of knowledge about dopamine neurotransmission has always been led by technical innovations. For example, Carlsson's discovery of brain dopamine in the 1950s was facilitated by the colorimetric detection of dopamine in tissue extracts. During the 1960s, subsequent studies of the enzymology and biochemistry of the dopamine pathway made use of new radiolabeled enzyme substrates, which allowed more sensitive detection of the rates of enzymatic product formation. New methods for the biochemical isolation of specific enzymes in the dopamine pathway also benefited from the availability of radioenzymatic assays. In the 1970s, classical chromatographic methods for the separation and detection of dopamine and its metabolites in biological extracts gave way to high-performance liquid chromatography (HPLC). When HPLC is coupled with electrochemical detection, quantities of dopamine as low as 10 pg can be detected. This innovation of sensitivity led in the 1980s to the development of cerebral microdialysis, in which dopamine and its metabolites are sampled from the interstitial fluid of the brain and their minute concentrations measured by HPLC at sampling intervals as low as 1 min. Still greater sensitivity can now be obtained by analytic methods employing mass spectroscopy or by using in vivo electrochemical detection.

Once the requirement of dopamine for normal movement had been established, the search for the dopaminergic neurons began. Dopamine and other monoamine neurotransmitters such as noradrenaline and serotonin characteristically form fluorescent adducts in the presence of formaldehyde vapor. Fluorescence histology of thin sections of brain proved to be extraordinarily useful for the mapping of monoamine neurons in the brain (Carlsson, Falck, & Hillarp 1962). Using this technique, a dozen clusters of fluorescent neurons

© Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
978-0-521-79002-4 - Imaging Dopamine
Paul Cumming
Excerpt
More information

were identified in the brainstem, midbrain, and hypothalamus. However, the fluorescence technique could not identify the precise neurochemical nature of these monoaminergic neurons.

Inflammation and loss of pigmented neurons in the mesencephalon had been described in patients dying of post-encephalitic parkinsonism (von Economo 1931), an influenza-like pandemic that arose toward the end of World War I. On the basis of fluorescence histology, the degenerating neurons of the mesencephalon were soon identified as catecholamine neurons. It is now understood that the formation of the black pigment neuromelanin in dopamine neurons of the substantia nigra is a by-product of dopamine synthesis in those cells. Soon after the connection between von Economo's encephalopathy and degeneration of dopamine neurons was established, dopamine depletion was reported in post mortem brain specimens from patients dying with Parkinson's disease (Ehringer & Hornykiewicz 1960). This observation led in short order to the first attempts at symptomatic treatment of Parkinson's disease with DOPA (Barbeau, Sourkes, & Murphy 1962). Thus, the early 1960s saw the awakening of the modern field of neurochemical pathology and of the rational treatment of a neurodegenerative disorder.

In the 1980s, a more general mapping of neurochemical anatomy was made possible based on immunohistochemistry. In this technique, antibodies are used for the localization in thin brain sections of specific antigens, such as enzymes in the pathway for dopamine synthesis. In parallel with the development of immunohistochemistry, autoradiographic methods made possible the mapping of neuroreceptors in vitro. Finally, in situ hybridization, molecular cloning techniques, and gene knockout technology all have contributed profoundly to studies of dopamine in recent decades.

In this biography of dopamine, the events and processes in the pathway for dopamine neurotransmission are described sequentially, beginning with the formation of tyrosine in the liver and ending with the mediation of post-synaptic signaling by dopamine receptors. At each step in the pathway, the relevant biochemistry is reviewed in detail and discussed in the context of quantitative PET studies. A basic knowledge of biochemistry and molecular biology is assumed on the part of the reader, and concepts relevant to the compartmental analysis of PET studies are introduced as each class of radiotracer is considered.

For good or for ill, PET reveals biochemical processes in the crucible of the living brain, as distinct from the biochemist's test tube. However, PET, and to an even greater extent SPET, suffers from inherent limitations, in addition to financial considerations. Molecular imaging techniques have low spatial resolution, meaning that tracer uptake in small brain structures cannot be detected

© Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
978-0-521-79002-4 - Imaging Dopamine
Paul Cumming
Excerpt
More information

without loss of information. In addition, temporal resolution is low, meaning that rapid dynamic processes are difficult to study, since one may require several hours in order to arrive at an equilibrium condition. Finally, process resolution is low, meaning that it can be difficult to identify the precise biological meaning of PET results. In addition to the usual problems (common to all pharmacology) presented by unspecific drugs, molecular imaging necessarily introduces the complexities of kinetic modeling. In this context, the objective of kinetic modeling is to employ a dynamic measurement of brain radioactivity concentrations for the calculation of a physiological parameter, such as blood flow, glucose consumption, or neuroreceptor binding. While kinetic modeling may seem an obstacle to newcomers in the field of dopamine imaging, most kinetic models can in fact be understood with some calculus. Unfortunately, there is presently no didactic reference textbook on kinetic modeling. While this book does not rectify the lack of a general introduction to radiotracer kinetics, compartmental models are presented diagrammatically, so as to be understood intuitively. Detailed exposition of the actual mathematics underlying specific modeling procedures can be found elsewhere.

In spite of the lack of mathematical presentation, the book is thematically all about modeling. The entire pathway for dopamine transmission in living brain can be compared to a model kit. The constituent parts have mostly been identified and their connectivity has been established. Results of PET studies, in conjunction with biochemical studies in vitro, have assigned magnitudes to the several components of the dopamine system. While the instructions for assembly are presently missing, it can be hoped that the coming decades will see the realization of a comprehensive model of dopamine transmission with inestimable heuristic value for the understanding of human disease. In addition, dopamine imaging is now enabling the development of a neurochemical typology, in which human personality and predispositions are understood in terms of neurochemical substrates.

© Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
978-0-521-79002-4 - Imaging Dopamine
Paul Cumming
Excerpt
More information

1

The life history of dopamine

1.1. A brief overview of the dopamine pathway

The life history of a dopamine molecule begins in the liver, with the synthesis of the precursor tyrosine, and ends in the kidney, with the elimination of the conjugated dopamine metabolites to the urine. Only during a brief and specific interval in its life can a dopamine molecule engage in its proper function, which is the mediation of signaling via activation of dopamine receptors. The chemical structures of molecules in the dopamine biosynthesis and catabolic pathway are illustrated in Figure 1.1. This scheme does not include the catecholamines noradrenaline and adrenaline, for which dopamine is a precursor, since these substances might properly serve as the topic of another book.

As presented in Figure 1.1, all the reactants and enzymes in the dopamine pathway seem to be present in the same space. However, in the living organism, molecules and chemical reactions normally occur within strictly segregated spaces, known as metabolic compartments. Transfer of a molecule in the dopamine pathway from one compartment to another may be strictly impeded by diffusion barriers, or may occur via carrier-mediated facilitated diffusion or by ATP-driven active transport. Thus, the schematic pathway for dopamine synthesis in the living organism should be projected onto a model containing cellular compartments. The model proposed by Carlsson (1966) illustrates the blood, extracellular space, intracellular space, and vesicles as distinct compartments (Figure 1.2). Enzymes and transporters conduct the transfer of mass from one compartment to another, here represented as arrows. The purpose of this book is to quantify the diverse processes implied in this diagram.

Tyrosine is formed in the liver from the essential amino acid phenylalanine by a specific hydroxylase (EC 1.14.16.1), or may be derived from dietary sources.

© Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
978-0-521-79002-4 - Imaging Dopamine
Paul Cumming
Excerpt
More information

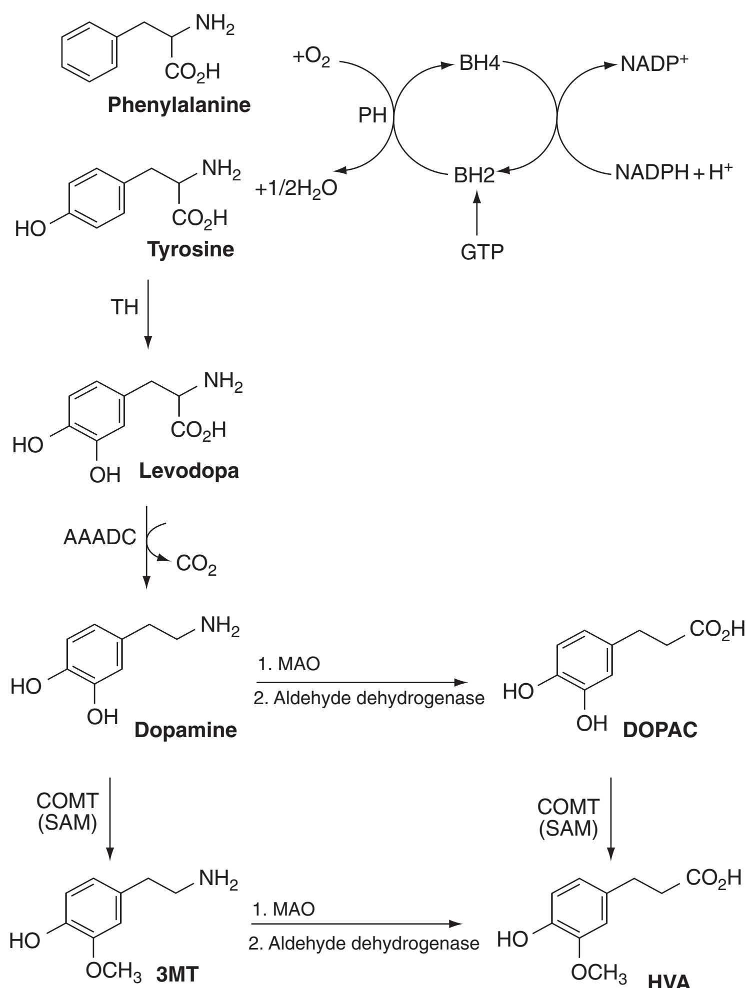

Figure 1.1 The pathway for the synthesis and catabolism of dopamine. PH, phenylalanine hydroxylase; BH2/BH4, dihydro- and tetrahydrobiopterin; TH, tyrosine hydroxylase; AAADC, aromatic amino acid decarboxylase; MAO, monoamine oxidase; COMT, catechol-*O*-methyltransferase; SAM, S-adenosylmethionine; 3MT, 3-methoxyryramine; DOPAC, dihydroxyphenylacetic acid; HVA, homovanillic acid.

Upon entry into the blood plasma compartment, tyrosine is delivered to the corporal tissues. Entry of tyrosine into the brain is impeded by the blood–brain barrier, which restricts access of many solutes to the brain. However, a facilitated diffusion carrier for tyrosine and other large neutral amino acids (LNAAs) is present in the capillary epithelium of the blood–brain barrier. The common carrier for LNNAs functions like a revolving door, transferring its substrates in and out of the brain. This process eventually establishes equilibrium between the blood and the

© Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
978-0-521-79002-4 - Imaging Dopamine
Paul Cumming
Excerpt
More information

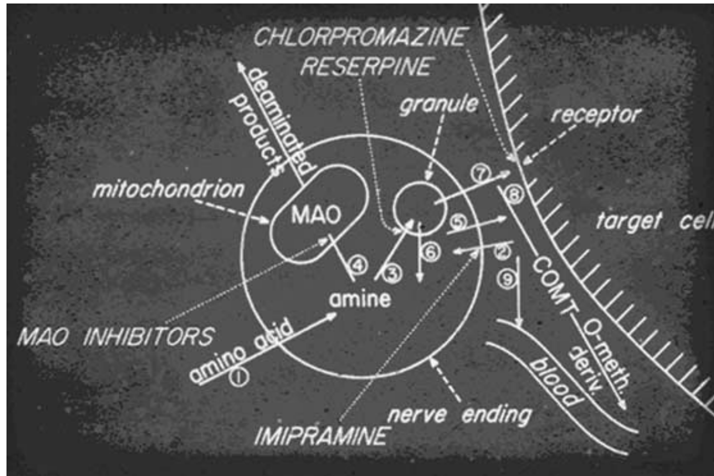

Figure 1.2 Schematic diagram of the dopamine synapse presented by Professor Arvid Carlsson in 1966. Processes in the pathway for dopamine are expressed as numbered arrows, including (1) entry of the amino acid precursor into the dopamine terminal, (2,5) bidirectional transfer of dopamine across the plasma membrane, (3) transfer of cytosolic dopamine into vesicles, and (4) oxidative deamination, and (7) binding at post-synaptic receptors. Reproduced with the kind permission of Professor Arvid Carlsson, University of Gothenburg. (For color version, see plate section.)

brain concentrations of tyrosine and its other substrates, with the key specification that concentration gradients cannot be generated by facilitated diffusion.

Once in the brain, tyrosine can be incorporated into proteins, or may serve as a precursor for the biosynthesis of DOPA within catecholamine neurons. This latter process is catalyzed by tyrosine hydroxylase (TH: EC 1.14.16.2). Under ordinary conditions, TH is nearly saturated with tyrosine in the brain; consequently, the rate of tyrosine hydroxylation is determined by the intrinsic activity of the enzyme, such that TH is classically considered the rate-limiting step for the synthesis of dopamine and other catecholamines. Regulation of TH activity is mediated by the changes in the affinity of TH for its rate-limiting co-substrate tetrahydrobiopterin (BH_4), which is modulated by phosphorylation of specific amino acid residues in the TH enzyme. In addition, dopamine and other end products, as well as synthetic amino acids, can modulate the activity of TH by simple competition at the catalytic site.

Several possible fates are available for cerebral DOPA, whether it is formed in situ from brain tyrosine, derived from circulation in the course of replacement therapy for Parkinson's disease, or while entering the brain as an exogenous substrate in the course of a positron emission tomography (PET) study. DOPA can be exported from the brain to blood via the common carrier for the LNAAs, or it can be a substrate for catechol-*O*-methyltransferase (COMT: EC 2.1.1.6) in

© Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
978-0-521-79002-4 - Imaging Dopamine
Paul Cumming
Excerpt
More information

the brain, yielding the inert metabolite *O*-methyldopa (OMD). The majority of DOPA formed within catecholamine neurons is normally decarboxylated by aromatic L-amino acid decarboxylase (AAADC: EC 4.1.1.28), yielding dopamine. However, the alternate metabolic fates for DOPA in the brain indicate that TH is not the sole and committed step in the synthesis of dopamine. Consequently, the modulation of AAADC activity can influence the branching ratio for DOPA metabolism, and therefore contribute to the overall regulation of dopamine synthesis.

Once formed in the cytosol or intracellular space of a dopamine neurone, dopamine is transferred by active transport into synaptic vesicles, which express the vesicular monoamine transporter type 2 (VMAT2). So long as dopamine is retained within the synaptic vesicle, it is protected from catabolism, but unbound cytosolic dopamine is rapidly metabolized by the successive actions of monoamine oxidase (MAO: EC 1.4.3.4) and COMT. This forked path of dopamine catabolism can proceed either way, although the major branch of dopamine metabolism in rat brain certainly proceeds first by oxidative deamination, yielding dihydroxyphenylacetic acid (DOPAC), which is subsequently *O*-methylated to yield homovanillic acid (HVA). Together, DOPAC and HVA are known as the acidic metabolites. A smaller fraction of dopamine in the brain is first *O*-methylated to produce 3-methoxytyramine (3MT), which is subsequently deaminated by MAO to yield HVA. The acidic metabolites are directly eliminated from the brain by a facilitated diffusion process that is inhibited by probenecid. Alternately, the acidic metabolites can be conjugated by aryl-sulfotransferase (EC 2.8.2.1) prior to diffusion from the brain into the cerebrospinal fluid (CSF) and thence to the bloodstream.

The active transport of cytosolic dopamine results in a very high concentration gradient across the vesicular membrane. The flux of dopamine into the vesicle is driven by a proton gradient established by the ATPase activity of VMAT2. A normally functioning dopamine vesicle thus resembles a mitochondrion acting in reverse; here, the consumption of ATP is used to maintain a proton gradient. The ATP-dependent dopamine storage is poisoned by the reserpine alkaloids, resulting in degranulation and massive efflux of stored dopamine into the cytosolic compartment. Because energy must be expended to maintain the dopamine concentration gradient across the vesicular membrane, degranulation is an early consequence of cerebral ischemia. Under normal physiological conditions, depolarization of the dopamine fiber encourages the fusion of a vesicle with the plasma membrane, accompanied by release of the vesicular contents into the interstitial fluid of the brain.

In the interstitial space at the site of vesicular fusion, the dopamine concentration is transiently very high, but rapidly declines as dopamine passively diffuses away from the fiber. In the striatum, interstitial dopamine is efficiently

© Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
978-0-521-79002-4 - Imaging Dopamine
Paul Cumming
Excerpt
More information

transferred back into the dopamine fiber, where it can once again run the gauntlet of MAO or be stored in vesicles for re-use. The plasma membrane dopamine transporter (DAT) facilitates the reuptake of interstitial dopamine back into the dopamine fiber. This transport process is driven by an inward sodium gradient across the polarized plasma membrane, but is not directly coupled to energy consumption. Cocaine, amphetamine, and other psychostimulants enhance the interstitial dopamine concentration in striatum by actions at the DAT. However, the relatively sparse dopamine innervation of the frontal cortex is poorly endowed with DAT, such that simple diffusion and the activity of COMT may have greater influence on interstitial dopamine concentrations. The consequent slower clearance of interstitial dopamine in the cortex results in a more prolonged action over a greater volume of tissue following an exocytosis event, a phenomenon known as volume transmission.

Once released into the interstitial space, dopamine can interact with two broad classes of G-protein-linked receptors, known as D_1-like and D_2-like receptors. In the striatum, both classes of receptors are located on medium spiny neurons, although D_2-like receptors also occur on presynaptic dopamine terminals, where they serve as "feedback" autoreceptors regulating dopamine synthesis and release. Whereas the functional aspects of dopamine D_1-like receptors are poorly understood, a great deal is known about D_2 receptors. For example, major beneficial effects of antiparkinsonian medications are mediated by activation of dopamine D_2 receptors. However, activation of dopamine D_2 receptors is a double-edged sword. Whereas it is abundantly clear that idiopathic Parkinson's disease is neurochemically a dopamine deficiency, the discovery that antipsychotic drugs antagonize D_2 receptors supports the hypothesis that schizophrenia is associated with a functional over-activity of these receptors.

Based on research during the past five decades, it has become possible to assign numbers or quantities to the arrows depicted in Figures 1.1 and 1.2. The activities of the enzymes catalyzing the steps in the pathway for dopamine synthesis and catabolism can be measured in biological samples, and the density of binding sites and transporters for dopamine can be quantified in membranes and thin sections from the brain. Corresponding assays can be obtained in living brain by measuring the uptake of radiolabeled tracers for dopamine synthesis and binding sites. In effect, PET is an extension of classical neurochemical techniques for quantifying aspects of the pathway for dopamine transmission, with the key distinction that PET results can be obtained non-invasively in the brain of living subjects, animal or human. As such, PET is a repudiation of the old canard that biochemists are "watch smashers," striving to understand a complex and subtle mechanism by first grinding it up with detergent in a pestle and mortar and then passing the resultant paste through a chromatographic column.

© Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
978-0-521-79002-4 - Imaging Dopamine
Paul Cumming
Excerpt
More information

1.2. A brief account of the blood–brain barrier

The brain is nourished by solutes arriving in the arterial blood and passing across the microvascular epithelium. Unlike the capillary cells of most other tissues, those in the brain form tight junctions, thus restricting the diffusion of molecules between the cerebral and the vascular compartments. Whereas proteins and most sugars are entirely restricted from diffusion across the barrier, gases such as oxygen and small lipophilic molecules can diffuse freely across the cells of the vascular epithelium. The tight junction is no barrier at all to water, which enters the brain on the arterial side and quickly returns to circulation on the venous side of the cerebral vasculature. Consequently, the water content of the brain is constantly being exchanged with "fresh" water derived from the plasma.

The blood–brain barrier is not just a physical barrier, but can actively exclude certain molecules from the brain by a process driven by the consumption of ATP. The extrusion of molecules from the brain is mediated by *p*-glycoprotein, which is a member of a large family of ATP-binding cassette transporters. The presence of this enzyme has been a major obstacle in the development of anti-HIV and antineoplastic compounds with activity in the central nervous system (Ebinger & Uhr 2006). The *p*-glycoprotein was originally understood to have a polarized distribution on the abluminal side of the capillary epithelium, but is now known to be expressed throughout the capillary cells and in perivascular astrocytes (Bendayan *et al.* 2006). Very recently, it has become possible to measure the concentration of *p*-glycoprotein in human brain using the PET ligand [^{11}C]verapamil (Bart *et al.* 2003). Administering cyclosporin to block the *p*-glycoprotein reveals that many PET radioligands are substantially extruded from the living brain (Ishiwata *et al.* 2007).

The selective permeability of the blood–brain barrier for DOPA and other large neutral amino acids relevant to the pathway for dopamine synthesis is mediated by a specific entity in the capillary epithelium known as the common transporter for the LNAAs. This protein is a member of a family of solute carriers; the sequence cloned from a rat tumor line predicts 12-transmembrane domains and an amino acid composition rich in cysteine residues (Kanai *et al.* 1998). The transporter permits the equilibration between plasma and brain of several substrates, including phenylalanine, tyrosine, DOPA, and OMD. Each of the ten or so endogenous LNAA substrates has an intrinsic permeability per unit surface area of the capillary bed, but in the living brain, the transporter is nearly saturated by the sum of its endogenous plasma substrates (Pardridge & Oldendorf 1977). Consequently, competition from other LNAAs in plasma generally reduces the influx of labeled amino acid for studies of dopamine synthesis in living brain.

© Cambridge University Press www.cambridge.org