# **48** Metabolism of the Nervous System

The nervous systems consists of various cell types. The most abundant cell in the nervous system is the glial cell, which consists of astrocytes and oligodendrocytes in the central nervous system, and Schwann cells in the peripheral nervous system. These cells provide support for the neurons and synthesize the protective myelin sheath that surrounds the axons emanating from the neurons. Microglial cells in the nervous system act as immune cells, destroying and ingesting foreign organisms that enter the nervous system. The interface between the brain parenchyma and the cerebrospinal fluid compartment is formed by the ependymal cells, which line the cavities of the brain and spinal cord. These cells use their cilia to allow for the circulation of the cerebrospinal fluid (CSF), which bathes the cells of the central nervous system.

The cells of the brain are separated from free contact with the rest of the body by the **blood-brain barrier**. The capillaries of the brain exhibit features, such as tight endothelial cell junctions, that restrict their permeability to metabolites in the blood. This protects the brain from compounds that might be toxic or otherwise interfere with nerve impulse transmission. It also affects the entry of precursors for brain metabolic pathways such as fuel metabolism and neurotransmitter synthesis.

Neurotransmitters can be divided structurally into two categories: **small nitrogen-containing neurotransmitters** and **neuropeptides**. The small nitrogencontaining neurotransmitters are generally synthesized in the **presynaptic terminal** from amino acids and intermediates of glycolysis and the TCA cycle. They are retained in storage vesicles until the neuron is depolarized. The **catecholamine** neurotransmitters (**dopamine**, **norepinephrine**, **and epinephrine**) are derived from tyrosine. **Serotonin** is synthesized from **tryptophan**. **Acetylcholine** is synthesized from **choline**, which can be supplied from the diet or is synthesized and stored as part of **phosphatidylcholine**. **Glutamate** and its neurotransmitter derivative,  $\gamma$ -aminobutyric acid (**GABA**), are derived from  $\alpha$ -ketoglutarate in the TCA cycle. **Glycine** is synthesized in the brain from **serine**. The synthesis of the neurotransmitters is regulated to correspond to the rate of depolarization of the individual neurons. A large number of cofactors are required for the synthesis of neurotransmitters, and deficiencies of **pyridoxal phosphate**, **thiaminepyrophosphate**, and **vitamin B12** result in a variety of neurologic dysfunctions.

Brain metabolism has a high requirement for glucose and oxygen. Deficiencies of either (hypoglycemia or hypoxia) affect brain function because they influence adenosine triphosphate (ATP) production and the supply of precursors for neurotransmitter synthesis. Ischemia elicits a condition in which increased calcium levels, swelling, glutamate excitotoxicity, and nitric oxide generation affect brain function, and can lead to a stroke. The generation of free radicals and abnormalities in nitric oxide production are important players in the pathogenesis of a variety of neurodegenerative diseases.

Because of the restrictions posed by the blood-brain barrier to the entry of a variety of substances into the central nervous system, the brain generally synthesizes and degrades its own lipids. Essential fatty acids can enter the brain, but the more common fatty acids do not. The turnover of lipids at the synaptic membrane is very rapid, and the neuron must replace those lipids lost during exocytosis. The glial cells produce the **myelin** sheath, which is composed primarily of lipids. These lipids are of a different composition than those of the neuronal cells. Because there is considerable lipid synthesis and turnover in the brain, this organ is sensitive to **disorders of peroxisomal function** (Refsum's disease; interference in very-long-chain fatty acid oxidation and  $\alpha$ -oxidation) and **lysosomal diseases** (mucopolysaccharidoses; inability to degrade complex lipids and glycolipids).



## THE WAITING ROOM

Katie Colamin, a 34-year-old dress designer, developed alarming palpitations of her heart while bending forward to pick up her cat. She also developed a pounding headache and sweated profusely. After 5 to 10 minutes, these symptoms subsided. One week later, her aerobic exercise instructor, a registered nurse, noted that Katie grew very pale and was tremulous during exercise. The instructor took Katie's blood pressure, which was 220 mm Hg systolic (normal, up to 120 at rest) and 132 mm Hg diastolic (normal, up to 80 at rest). Within 15 minutes, Katie recovered, and her blood pressure returned to normal. The instructor told Katie to see her physician the next day.

The doctor told Katie that her symptom complex coupled with severe hypertension strongly suggested the presence of a tumor in the medulla of one of her adrenal glands (a pheochromocytoma) that was episodically secreting large amounts of catecholamines, such as norepinephrine (noradrenaline) and epinephrine (adrenaline). Her blood pressure was normal until moderate pressure to the left of her umbilicus caused Katie to suddenly develop a typical attack, and her blood pressure rose rapidly. She was immediately scheduled for a magnetic resonance imaging (MRI) study of her adrenal glands. The MRI showed a  $3.5 \times 2.8 \times 2.6$  cm mass in the left adrenal gland, with imaging characteristics typical of a pheochromocytoma.

**Ivan Applebod's** brother, **Evan Applebod**, was 6 feet tall and weighed 425 pounds. He had only been successful in losing weight once in his life, in 1977. Evan's weight was not usually a concern for him, but in 1997 he had become concerned when it became difficult for him to take walks or go fishing because of joint pain in his knees. He was also suffering from symptoms suggestive of a peripheral neuropathy, manifest primarily as tingling in his legs. He had failed in all previous dieting attempts and was desperate now to lose weight. The physician placed Evan on a new drug, Redux, which had just been approved for use as a weight loss agent, and a slightly restricted low-fat, low-calorie diet. In 4 months, Evan's weight dropped from 425 pounds to 335 pounds, his total cholesterol dropped from 250 to 185, and his serum triglycerides dropped from 375 to 130. However, Redux was withdrawn from the market by its manufacturer late in 1997 because of its toxicity. Evan was then placed on Prozac, a drug used primarily as an antidepressant and less commonly as an appetite suppressant.

## I. CELL TYPES OF THE NERVOUS SYSTEM

The nervous system consists of neurons, the cells that transmit signals, and supporting cells, the neuroglia. The neuroglia consists of oligodendrocytes and astrocytes (collectively known as glial cells), microglial cells, ependymal cells, and Schwann cells. The neuroglia are designed to support and sustain the neurons and do so by surrounding neurons and holding them in place, supplying nutrients and oxygen to the neurons, insulating neurons so their electrical signals are more rapidly propagated, and cleaning up any debris that enters the nervous system. The central nervous system (CNS) consists of the brain and spinal cord. This system integrates all signals emanating from the peripheral nervous system (PNS). The PNS is composed of all neurons lying outside of the CNS.

#### A. Neurons

Neurons consist of a cell body (soma) from which long (axons) and short (dendrites) extensions protrude. Dendrites receive information from the axons of other neurons, whereas the axons transmit information to other neurons. The axon–dendrite connection is known as a synapse (Fig. 48.1). Most neurons contain multiple dendrites, each of which can receive signals from multiple axons. This configuration allows a single neuron to integrate information from multiple sources. Although neurons also contain just one axon, most axons branch extensively and distribute information to multiple targets (divergence). The neurons transmit signals by changes in the electrical potential across their membrane. Signaling across a synapse requires the release of neurotransmitters that, when bound to their specific receptors, initiate an electrical signal in the receiving or target cell. Neurons are terminally differentiated cells and, as such, have little capability for division. Because of this, injured neurons have a limited capacity to repair themselves and frequently undergo apoptosis (programmed cell death) when damaged.

#### B. Neuroglial Cells

#### 1. ASTROCYTES

The astrocytes are found in the CNS and are star-shaped cells that provide physical and nutritional support for neurons. During development of the CNS, the astrocytes guide neuronal migration to their final adult position and form a matrix that keeps neurons in place. These cells serve several functions, including the ability to phagocytose debris left behind by cells, to provide lactate (from glucose metabolism) as



**Fig. 48.1.** A neuron consists of a cell body (soma) with short extensions (dendrites) and a long extension (axon). The axon–dendrite interface is the synapse. A soma may receive input from multiple axons.

a carbon source for the neurons, and to control the brain extracellular ionic environment Astrocytes help to regulate the content of the extracellular fluid (ECF) by taking up, processing, and metabolizing nutrients and waste products.

#### 2. OLIGODENDROCYTES

The oligodendrocyte provides the myelin sheath that surrounds the axon, acting as an "insulation" for many of the neurons in the CNS. The myelin sheath consists of a lipid–protein covering of the axons (see section V.B. for a description of the composition and synthesis of the myelin sheath). Oligodendrocytes can form myelin sheaths around multiple neurons in the CNS by sending out processes that bind to the axons on target neurons. The speed with which a neuron conducts its electrical signal (action potential) is directly proportional to the degree of myelination. Oligodendrocytes, along with the astrocytes, form a supporting matrix for the neurons. Oligodendrocytes have a limited capacity for mitosis, and if damaged, do not replicate. If this occurs, demyelination of the axons may occur, resulting in abnormalities in signal conduction along that axon (see Biochemical Comments).

#### 3. SCHWANN CELLS

Schwann cells are the supporting cells of the PNS. Like oligodendrocytes, Schwann cells form myelin sheaths around the axons, but unlike the oligodendrocytes, Schwann cells only myelinate one axon. Schwann cells also clean up cellular debris in the PNS.

#### 4. MICROGLIAL CELLS

These are the smallest type of glial cells in the nervous system. They serve as immunologically responsive cells similar to the action of macrophages in the circulation. Microglial cells destroy invading microorganisms and phagocytose cellular debris.

#### 5. EPENDYMAL CELLS

The ependymal cells are ciliated cells that line the cavities (ventricles) of the CNS and the spinal cord. In some areas of the brain, the ependymal cells are functionally specialized to elaborate and secrete cerebrospinal fluid (CSF) into the ventricular system. The beating of the ependymal cilia allow for efficient circulation of the CSF throughout the CNS. The CSF acts as both a shock absorber protecting the CNS from mechanical trauma and a system for the removal of metabolic wastes. The CSF can be aspirated from the spinal canal and analyzed to determine whether disorders of CNS function, with their characteristic CSF changes, are present.

#### **II. THE BLOOD-BRAIN BARRIER**

#### A. Capillary Structure

In the capillary beds of most organs, a rapid passage of molecules occurs from the blood through the endothelial wall of the capillaries into the interstitial fluid. Thus, the composition of interstitial fluid resembles that of blood, and specific receptors or transporters in the plasma membrane of the cells being bathed by the interstitial fluid may directly interact with amino acids, hormones, or other compounds from the blood. In the brain, transcapillary movement of substrates in the peripheral circulation into the brain is highly restricted by the blood-brain barrier. This barrier limits the accessibility of blood-borne toxins and other potentially harmful compounds to the neurons of the CNS.

Peripheral axons can regenerate if Schwann cells are available to guide the growth of the axon. There is a synergistic interaction between the Schwann cells, secreted growth factors, and the axon that allows the axons to reconnect to its appropriate target.



## For many years, it had been believed that damaged neurons in

the CNS could not regenerate, for it was thought that there were no pluripotent stem cells (cells that could differentiate into various cell types found in the CNS) in the CNS. However, recent data suggest that cells found within the ependymal layer can act as neural stem cells, which under appropriate stimulation can regenerate neurons. Such a finding opens up a large number of potential treatments for diseases that alter neuronal cell function.

The blood-brain barrier begins with the endothelial cells that form the inner lining of the vessels supplying blood to the CNS (Fig. 48.2). Unlike the endothelial cells of other organs, these cells are joined by tight junctions that do not permit the movement of polar molecules from the blood into the interstitial fluid bathing the neurons. They also lack mechanisms for transendothelial transport that are present in other capillaries of the body. These mechanisms include fenestrations ("windows" or pores that span the endothelial lining and permit the rapid movement of molecules across membranes) or transpinocytosis (vesicular transport from one side of the endothelial cell to another).

The endothelial cells actively, as well as passively, serve to protect the brain. Because they contain a variety of drug-metabolizing enzyme systems similar to the drug-metabolizing enzymes found in the liver, the endothelial cells can metabolize neurotransmitters and toxic chemicals and, therefore, form an enzymatic barrier to entry of these potentially harmful substances into the brain. They actively pump hydrophobic molecules that diffuse into endothelial cells back into the blood (especially xenobiotics) with P-glycoproteins, which act as transmembranous, ATPdependent efflux pumps. Although lipophilic substances, water, oxygen, and carbon dioxide can readily cross the blood-brain barrier by passive diffusion, other molecules depend on specific transport systems. Differential transporters on the luminal and abluminal endothelial membranes can transport compounds into, as well as out of, the brain.

Further protection against the free entry of blood-borne compounds into the CNS is provided by a continuous collagen-containing basement membrane that completely surrounds the capillaries. The basement membrane appears to be surrounded by the foot processes of astrocytes. Thus, compounds must pass through endothelial cell membranes, the enzymatic barrier in the endothelial cells, the basement membrane, and possibly additional cellular barriers formed by the astrocytes to reach the neurons in the brain.

#### B. Transport through the Blood-Brain Barrier

Many nonpolar substances, such as drugs and inert gases, probably diffuse through the endothelial cell membranes. A large number of other compounds are transported through the endothelial capillaries by facilitative transport, whereas others, such as nonessential fatty acids, cannot cross the blood-brain barrier. Essential fatty acids, however, are transported across the barrier.

## 1. FUELS

Glucose, which is the principle fuel of the brain, is transported through both endothelial membranes by facilitated diffusion via the GLUT-1 transporter (see Fig. 27.4). GLUT-3 transporters present on the neurons then allow the neurons to transport the glucose from the ECF. Glial cells express GLUT-1 transporters. Although the rate of glucose transport into the ECF normally exceeds the rate required for energy metabolism by the brain, glucose transport may become rate-limiting as blood glucose levels fall below the normal range. Thus, individuals begin to experience hypoglycemic symptoms at approximately 60 mg/dL, as the glucose levels are reduced to the K<sub>m</sub>, or below the K<sub>m</sub> values of the GLUT-1 transporters in the endothelial cells of the barrier.

Monocarboxylic acids, including L-lactate, acetate, pyruvate, and the ketone bodies acetoacetate and  $\beta$ -hydroxybutyrate, are transported by a separate stereospecific system that is slower than the transport system for glucose. During starvation, when the level of ketone bodies in the blood is elevated, this transporter is upregulated. Ketone bodies are important fuels for the brain of both the adult and the neonate during prolonged starvation (greater than 48 hours).



Fig. 48.2. The blood-brain barrier. Compounds in the blood cannot freely pass into the brain; they must traverse the endothelial cells, basement membrane, and astrocytes, by use of specific carriers to gain access to the brain. Very lipophilic molecules may pass through all of these membranes in the absence of a carrier.



A number of disorders of glucose transport across the blood-brain barrier are known. The most common of these is facilitated glucose transporter protein type 1 (GLUT1) deficiency syndrome. In this disorder, GLUT1 transporters are impaired, which results in a low glucose concentration in the CSF (a condition known as hypoglycorrhachia). A diagnostic indication of this disorder is that in the presence of normal blood glucose levels the ratio of CSF glucose to blood glucose levels is less than 0.4. Clinical features are variable but include seizures, developmental delay, and a complex motor disorder. These symptoms are the result of inadequate glucose levels within the brain. The disorder can be treated by prescribing a ketogenic diet (highfat, low-carbohydrate). This will force the patient to produce ketone bodies, which are easily transported into the CNS and can spare the brain's requirement for glucose as an energy source.



The finding that the large, neutral amino acids (LNAA) have a common carrier system across the

blood-brain barrier suggests that if one amino acid is in excess, it can, by competitive inhibition, result in a lower transport of the other amino acids. This suggests that the mental retardation that results from untreated PKU and maple syrup urine disease (see Chapter 39) may be attributable to the high levels of either phenylalanine or branched-chain amino acids in the blood. These high levels overwhelm the LNAA carrier, such that excessive levels of the damaging amino acid enter the CNS. In support of this theory is the finding that treatment of PKU patients with large doses of LNAA lacking phenylalanine resulted in a decrease of phenylalanine levels in the CSF and brain, with an improvement in their cognitive functions as well.

#### 2. AMINO ACIDS AND VITAMINS

Large neutral amino acids (LNAA, such as phenylalanine, leucine, tyrosine, isoleucine, valine, tryptophan, methionine, and histidine) rapidly enter the CSF via a single amino acid transporter. (L-[leucine preferring]-system amino acid transporter). Many of these compounds are essential in the diet and must be imported for protein synthesis or as precursors of neurotransmitters. Because a single transporter is involved, these amino acids compete with each other for transport into the brain.

The entry of small neutral amino acids, such as alanine, glycine, proline, and  $\gamma$ aminobutyrate (GABA), is markedly restricted because their influx could dramatically change the content of neurotransmitters (see section III). They are synthesized in the brain, and some are transported out of the CNS and into the blood via the A-(alanine-preferring)-system carrier. Vitamins have specific transporters through the blood-brain barrier as they do in most tissues.

#### 3. RECEPTOR-MEDIATED TRANSCYTOSIS

Certain proteins, such as insulin, transferrin, and insulin-like growth factors, cross the blood-brain barrier by receptor-mediated transcytosis. Once the protein binds to its membrane receptor, the membrane containing the receptor-protein complex is endocytosed into the endothelial cell to form a vesicle. It is released on the other side of the endothelial cell. Absorptive-mediated transcytosis also can occur. It differs from receptor-mediated transcytosis in that the protein binds nonspecifically to the membrane and not to a distinct receptor.

## III. SYNTHESIS OF SMALL NITROGEN-CONTAINING NEUROTRANSMITTERS

Molecules that serve as neurotransmitters fall into two basic structural categories: (1) small nitrogen-containing molecules, and (2) neuropeptides. The major small nitrogen-containing molecule neurotransmitters include glutamate,  $\gamma$ -aminobutyric acid (GABA), glycine, acetylcholine, dopamine, norepinephrine, serotonin, and histamine. Additional neurotransmitters that fall into this category include epinephrine, aspartate, and nitric oxide. In general, each neuron synthesizes only those neurotransmitters that it uses for transmission through a synapse or to another cell. The neuronal tracts are often identified by their neurotransmitter; for example, a dopaminergic tract synthesizes and releases the neurotransmitter dopamine.

Neuropeptides are usually small peptides, which are synthesized and processed in the CNS. Some of these peptides have targets within the CNS (such as endorphins, which bind to opioid receptors and block pain signals), whereas others are released into the circulation to bind to receptors on other organs (such as growth hormone and thyroid-stimulating hormone). Many neuropeptides are synthesized as a larger precursor, which is then cleaved to produce the active peptides. Until recently, the assumption was that a neuron only synthesized and released a single neurotransmitter. More recent evidence suggests that a neuron may contain (1) more than one small molecule neurotransmitter, (2) more than one neuropeptide neurotransmitter, or (3) both types of neurotransmitters. The differential release of the various neurotransmitters is the result of the neuron altering its frequency and pattern of firing.

#### A. General Features of Neurotransmitter Synthesis

A number of features are common to the synthesis, secretion, and metabolism of most small nitrogen-containing neurotransmitters (Table 48.1). Most of these neurotransmitters are synthesized from amino acids, intermediates of glycolysis and the TCA cycle, and  $O_2$  in the cytoplasm of the presynaptic terminal. The rate of synthesis is

#### Table 48.1. Features Common to Neurotransmitters<sup>a</sup>

- Synthesis from amino acid and common metabolic precursors usually occurs in the cytoplasm of the presynaptic nerve terminal. The synthetic enzymes are transported by fast axonal transport from the cell body, where they are synthesized, to the presynaptic terminal.
- The synthesis of the neurotransmitter is regulated to correspond to the rate of firing of the neuron, both acutely and through long-term enhancement of synaptic transmission.
- The neurotransmitter is actively taken up into storage vesicles in the presynaptic terminal.
- The neurotransmitter acts at a receptor on the postsynaptic membrane.
- The action of the neurotransmitter is terminated through reuptake into the presynaptic terminal, diffusion away from the synapse, or enzymatic inactivation. The enzymatic inactivation may occur in the postsynaptic terminal, the presynaptic terminal, or an adjacent astrocyte or microglial cell.
- The blood-brain barrier affects the supply of precursors for neurotransmitter synthesis.

<sup>a</sup>Not all neurotransmitters exhibit all of these features. Nitric oxide is an exception to most of these generalities. Some neurotransmitters (epinephrine, serotonin, and histamine) are also secreted by cells other than neurons. Their synthesis and secretion by non-neuronal cells follows other principles.

generally regulated to correspond to the rate of firing of the neuron. Once synthesized, the neurotransmitters are transported into storage vesicles by an ATP-requiring pump linked with the proton gradient. Release from the storage vesicle is triggered by the nerve impulse that depolarizes the postsynaptic membrane and causes an influx of  $Ca^{2+}$  ions through voltage-gated calcium channels. The influx of  $Ca^{2+}$  promotes fusion of the vesicle with the synaptic membrane and release of the neurotransmitter into the synaptic cleft. The transmission across the synapse is completed by binding of the neurotransmitter to a receptor on the postsynaptic membrane (Fig. 48.3).

The action of the neurotransmitter is terminated through reuptake into the presynaptic terminal, uptake into glial cells, diffusion away from the synapse, or enzymatic inactivation. The enzymatic inactivation may occur in the postsynaptic terminal, the presynaptic terminal or an adjacent astrocyte microglia cell, or in endothelial cells in the brain capillaries. Drugs have been developed that block neurotransmitter uptake into storage vesicles. Reserpine, which blocks catecholamine uptake into vesicles, had been used as an antihypertensive and antiepileptic drug for many years, but it was noted that a small percentage of patients on the drug became depressed and even suicidal. Animals treated with reserpine showed signs of lethargy and poor appetite, similar to depression in humans. Thus, a link was forged between monoamine release and depression in humans.



Fig. 48.3. Action of neurotransmitters.

Not all neurotransmitters exhibit all of these features. Nitric oxide, because it is a gas, is an exception to most of these generalities. Some neurotransmitters are synthesized and secreted by both neurons and other cells (e.g., epinephrine, serotonin, histamine).

#### B. Dopamine, Norepinephrine, and Epinephrine

#### SYNTHESIS OF THE CATECHOLAMINE NEUROTRANSMITTERS 1.

These three neurotransmitters are synthesized in a common pathway from the amino acid L-tyrosine. Tyrosine is supplied in the diet or is synthesized in the liver from the essential amino acid phenylalanine by phenylalanine hydroxylase (see Chapter 39). The pathway of catecholamine biosynthesis is shown in Figure 48.4.

The first and rate-limiting step in the synthesis of these neurotransmitters from tyrosine is the hydroxylation of the tyrosine ring by tyrosine hydroxylase, a tetrahydrobiopterin (BH<sub>4</sub>)-requiring enzyme. The product formed is dihydroxyphenylalanine or DOPA. The phenyl ring with two adjacent OH groups is a catechol, and hence dopamine, norepinephrine, and epinephrine are called catecholamines.

The second step in catecholamine synthesis is the decarboxylation of DOPA to form dopamine. This reaction, like many decarboxylation reactions of amino acids, requires pyridoxal phosphate. Dopaminergic neurons (neurons using dopamine as a neurotransmitter) stop the synthesis at this point, because these neurons do not synthesize the enzymes required for the subsequent steps.

Neurons that secrete norepinephrine synthesize it from dopamine in a hydroxylation reaction catalyzed by dopamine β-hydroxylase (DBH). This enzyme is present only within the storage vesicles of these cells. Like tyrosine hydroxylase, it is a mixed-function oxidase that requires an electron donor. Ascorbic acid (vitamin C) serves as the electron donor and is oxidized in the reaction. Copper  $(Cu^{2+})$  is a bound cofactor required for the electron transfer.

Although the adrenal medulla is the major site of epinephrine synthesis, it is also synthesized in a few neurons that use epinephrine as a neurotransmitter. These neurons contain the above pathway for norepinephrine synthesis and in addition contain the enzyme that transfers a methyl group from SAM to norepinephrine to form epinephrine. Thus, epinephrine synthesis is dependent on the presence of adequate levels of B12 and folate (see Chapter 40).

#### STORAGE AND RELEASE OF CATECHOLAMINES 2.

Ordinarily, only low concentrations of catecholamines are free in the cytosol, whereas high concentrations are found within the storage vesicles. Conversion of tyrosine to L-DOPA and that of L-DOPA to dopamine occurs in the cytosol. Dopamine is then taken up into the storage vesicles. In norepinephrine-containing neurons, the final  $\beta$ -hydroxylation reaction occurs within the vesicles.

The catecholamines are transported into vesicles by the protein VMAT2 (vesicle membrane transporter 2) (Fig. 48.5). The vesicle transporters contain 12 transmembrane domains and are homologous to a family of bacterial drug resistance transporters, including the P-glycoprotein. The mechanism that concentrates the catecholamines in the storage vesicles is an ATP-dependent process linked to a proton pump (secondary active transport). Protons are pumped into the vesicles by a vesicular-ATPase (v-ATPase). The protons then exchange for the positively charged catecholamine via the transporter VMAT2. The influx of the catecholamine is thus driven by the H<sup>+</sup> gradient across the membrane. The intravesicular concentration of catecholamines is approximately 0.5 M, roughly 100 times the cytosolic concentration. In the vesicles, the catecholamines exist in a complex with ATP and acidic proteins known as chromogranins.



Chromogranins are required for the biogenesis of the secretory vesicle. When released from the vesicle, chromogranins can be proteolytically clipped to form bioactive peptides. Elevated levels of chromogranins in the circulation may be found in patients harboring neuroendocrine tumors, such as a pheochromocytoma.



Fig. 48.4. The pathways of catecholamine and melanin biosynthesis. PLP = pyridoxal phosphate.  $BH_4 = tetrahydrobiopterin$ . The shaded boxes indicate the enzymes, which, when defective, lead to albinism.

In albinism, either the copperdependent tyrosine hydroxylase of melanocytes (which is distinct from the tyrosine hydroxylase found in the adrenal medulla) or other enzymes that convert tyrosine to melanins may be defective. Individuals with albinism suffer from a lack of pigment in the skin, hair, and eyes, and they are sensitive to sunlight.



Fig. 48.5. Transport of catecholamines into storage vesicles. This is a secondary active transport based on the generation of a proton gradient across the vesicular membrane. NT+ = positively charged neurotransmitter (catecholamine);  $DBH = dopamine \beta$ -hydroxylase; VMAT2 = vesicle membrane transporter 2; V-ATPase = vesicular ATPase.



Tyramine is a degradation product of tyrosine that can lead to headaches, palpitations, nausea and vomiting, and elevated blood pressure if

present in large quantities. Tyramine mimics norepinephrine and binds to norepinephrine receptors, stimulating them. Tyramine is inactivated by MAO-A, but if a person is taking an MAO inhibitor, foods containing tyramine should be avoided.



The vesicles play a dual role: they maintain a ready supply of catecholamines at the nerve terminal that is available for immediate release, and they mediate the process of release. When an action potential reaches the nerve terminal, Ca<sup>2+</sup> channels open, allowing an influx of Ca<sup>2+</sup>, which promotes the fusion of vesicles with the neuronal membrane. The vesicles then discharge their soluble contents, including the neurotransmitters, ATP, chromogranins, and DBH, into the extraneuronal space by the process of exocytosis. In some cases, the catecholamines affect other neurons. In other cases, they circulate in the blood and initiate responses in peripheral tissues.

#### INACTIVATION AND DEGRADATION OF CATECHOLAMINE 3. **NEUROTRANSMITTERS**

The action of catecholamines is terminated through reuptake into the presynaptic terminal and diffusion away from the synapse. Degradative enzymes are present in the presynaptic terminal, and in adjacent cells, including glial cells and endothelial cells.

Two of the major reactions in the process of inactivation and degradation of catecholamines are catalyzed by monamine oxidase (MAO) and catechol-O-methyltransferase (COMT). MAO is present on the outer mitochondrial membrane of many cells and oxidizes the carbon containing the amino group to an aldehyde, thereby releasing ammonium ion (Fig. 48.6). In the presynaptic terminal, MAO inactivates catecholamines that are not protected in storage vesicles. (Thus, drugs that deplete storage vesicles indirectly increase catecholamine degradation.) There are two isoforms of MAO with different specificities of action: MAO-A preferentially deaminates norepinephrine and serotonin, whereas MAO-B acts on a wide spectrum of phenylethylamines (phenylethyl refers to a -CH<sub>2</sub>- group linked to a phenyl ring). MAO in the liver and other sites protects against the ingestion of dietary biogenic amines such as the tyramine found in many cheeses.

COMT is also found in many cells, including the erythrocyte. It works on a broad spectrum of extraneuronal catechols and those that have diffused away from the synapse. COMT transfers a methyl group from SAM to a hydroxyl group on the catecholamine or its degradation product (see Fig. 48.6). Because the inactivation reaction requires SAM, it is indirectly dependent on vitamins B12 and folate. The action of MAO and COMT can occur in almost any order, thereby resulting in a large number of degradation products and intermediates, many of which appear in the urine. Cerebrospinal homovanillylmandelic acid (HVA) is an indicator of dopamine degradation. Its concentration is decreased in the brain of patients with Parkinson's disease.

Katie Colamin's doctor ordered plasma catecholamine (epinephrine, norepinephrine, and dopamine) levels and also had Katie collect a 24-hour urine specimen for the determination of catecholamines and their degradation products. All of these tests showed unequivocal elevations of these compounds in Katie's blood and urine. Katie was placed on phenoxybenzamine, an  $\alpha_1$ - and  $\alpha_2$ -adrenergic receptor antagonist that blocks the pharmacologic effect of the elevated catecholamines on these receptors. After ruling out evidence to suggest metastatic disease to the liver or other organs (in case Katie's tumor was malignant), the doctor referred Katie to a surgeon with extensive experience in adrenal surgery.

The catecholamines exert their physiologic and pharmacologic effects by circulating in the bloodstream to target cells whose plasma membranes contain catecholamine receptors. This interaction initiates a biochemical cascade leading to responses that are specific for different types of cells. Patients such as Katie Colamin experience palpitations, excessive sweating, hypertensive headaches, and a host of other symptoms when a catecholamine-producing tumor of the adrenal medulla suddenly secretes supraphysiologic amounts of epinephrine or norepinephrine into the venous blood draining the neoplasm.



**Fig. 48.6.** Inactivation of catecholamines. Methylation and oxidation may occur in any order. Methylated and oxidized derivatives of norepinephrine and epinephrine are produced, and 3-methoxy-4-hydroxymandelic acid is the final product. These compounds are excreted in the urine. MAO = monoamine oxidase; COMT = catechol O-methyltransferase; SAM = S-adenosylmethionine; SAH = S-adenosylhomocysteine.

#### 4. REGULATION OF TYROSINE HYDROXYLASE

Efficient regulatory mechanisms coordinate the synthesis of catecholamine neurotransmitters with the rate of firing. Tyrosine hydroxylase, the first committed step and rate-limiting enzyme in the pathway, is regulated by feedback inhibition that is coordinated with depolarization of the nerve terminal. Tyrosine hydroxylase is inhibited by free cytosolic catecholamines that compete at the binding site on the enzyme for the pterin cofactor (tetrahydrobiopterin,  $BH_4$ ; see Chapter 39).

Depolarization of the nerve terminal activates tyrosine hydroxylase. Depolarization also activates a number of protein kinases (including protein kinase C, protein kinase A [the cAMP-dependent protein kinase] and CAM kinases [Ca<sup>2+</sup>-calmod-ulin–dependent kinases]) that phosphorylate tyrosine hydroxylase. These activation steps result in an enzyme that binds  $BH_4$  more tightly, making it less sensitive to end-product inhibition.

In addition to these short-term regulatory processes, a long-term process involves alterations in the amounts of tyrosine hydroxylase and dopamine  $\beta$ -hydroxylase present in nerve terminals. When sympathetic neuronal activity is increased for a prolonged period, the amounts of mRNA coding for tyrosine hydroxylase and dopamine  $\beta$ -hydroxylase are increased in the neuronal perikarya (the cell body of the neuron). The increased gene transcription may be the result of phosphorylation of CREB (cAMP response element binding protein; see Chapter 26) by

In addition to the catecholamines, serotonin is also inactivated by monoamine oxidase. The activity of a number of antipsychotic drugs are based on inhibiting MAO. The first generation of drugs (exemplified by iproniazid, which was originally developed as an antituberculosis drug and was found to induce mood swings in patients) were irreversible inhibitors of both the A and B forms of MAO. Although leading to a reduction in the severity of depression (by maintaining higher levels of serotonin), these drugs suffered from the "cheese" effect. Cheese and other foods that are processed over long periods (such as red wine) contain tyramine. Usually tyramine is inactivated by MAO-A, but if an individual is taking an MAO inhibitor, tyramine levels will increase. Tyramine induces the release of norepinephrine from storage vesicles, which leads to potentially life-threatening hypertensive episodes. When it was realized that MAO existed in two forms, selective irreversible inhibitors were developed; examples include clorgyline for MAO-A, and deprenyl for MAO-B. Deprenyl has been used to treat Parkinson's disease (which is caused by a lack of dopamine, which is also inactivated by MAO). Deprenyl, however, is not an antidepressant. Clorgyline is an antidepressant but suffers from the "cheese" effect. This led to the development of the third generation of MAO inhibitors, which are reversible inhibitors of the enzyme, as typified by moclobemide. Moclobemide is a specific, reversible inhibitor of MAO-A and is effective as an antidepressant. More importantly, because of the reversible nature of the drug, the "cheese" effect is not observed, because as tyramine levels increase, they displace the drug from MAO, and the tyramine is safely inactivated.

protein kinase A or by other protein kinases. CREB then binds to the CRE (cAMP response element) in the promoter region of the gene (similar to the mechanism for the induction of gluconeogenic enzymes in the liver). The newly synthesized enzyme molecules are then transported down the axon to the nerve terminals. The concentration of dopamine decarboxylase in the terminal does not appear to change in response to neuronal activity.

#### C. Metabolism of Serotonin

The pathway for the synthesis of serotonin from tryptophan is very similar to the pathway for the synthesis of norepinephrine from tyrosine (Fig. 48.7). The first enzyme of the pathway, tryptophan hydroxylase, uses an enzymic mechanism similar to that of tyrosine and phenylalanine hydroxylase and requires  $BH_4$  to hydroxylate the ring structure of tryptophan. The second step of the pathway is a decarboxylation reaction



Fig. 48.7. Synthesis and inactivation of serotonin.

catalyzed by the same enzyme that decarboxylates DOPA. Serotonin, like the catecholamine neurotransmitters, can be inactivated by MAO.

The neurotransmitter melatonin is also synthesized from tryptophan (see Fig. 48.7). Melatonin is produced in the pineal gland in response to the light-dark cycle, its level in the blood rising in a dark environment. It is probably through melatonin that the pineal gland conveys information about light-dark cycles to the body, organizing seasonal and circadian rhythms. Melatonin also may be involved in regulating reproductive functions.

#### D. Metabolism of Histamine

Within the brain, histamine is produced both by mast cells and by certain neuronal fibers. Mast cells are a family of bone marrow-derived secretory cells that store and release high concentrations of histamine. They are prevalent in the thalamus, hypothalamus, dura mater, leptomeninges, and choroid plexus. Histaminergic neuronal cell bodies in the human are found in the tuberomamillary nucleus of the posterior basal hypothalamus. The fibers project into nearly all areas of the CNS, including the cerebral cortex, the brainstem, and spinal cord.

Histamine is synthesized from histidine in a single enzymatic step. The enzyme histidine decarboxylase requires pyridoxal phosphate, and its mechanism is very similar to that of DOPA decarboxylase (Fig. 48.8).

Like other neurotransmitters, newly synthesized neuronal histamine is stored within the nerve terminal vesicle. Depolarization of nerve terminals activates the exocytotic release of histamine by voltage-dependent as well as a calcium-dependent mechanism.

Once released from neurons, histamine is thought to activate both postsynaptic and presynaptic receptors. Unlike other neurotransmitters, histamine does not appear to be recycled into the presynaptic terminal to any great extent. However, astrocytes have a specific high-affinity uptake system for histamine and may be the major site of the inactivation and degradation of this monoamine.

The first step in the inactivation of histamine in the brain is methylation (see Fig. 48.8). The enzyme histamine methyltransferase transfers a methyl group from SAM to a ring nitrogen of histamine to form methylhistamine. The second step is oxidation by MAO-B, followed by an additional oxidation step. In peripheral tissues, histamine undergoes deamination by diamine oxidase, followed by oxidation to a carboxylic acid (see Fig. 48.8).

## E. Acetylcholine

#### **SYNTHESIS** 1.

The synthesis of acetylcholine from acetyl CoA and choline is catalyzed by the enzyme choline acetyltransferase (ChAT) (Fig. 48.9). This synthetic step occurs in the presynaptic terminal. The compound is stored in vesicles and later released through calcium-mediated exocytosis. Choline is taken up by the presynaptic terminal from the blood via a low-affinity transport system (high K<sub>m</sub>) and from the synaptic cleft via a high-affinity transport mechanism (low K<sub>m</sub>). It is also derived from the hydrolysis of phosphatidylcholine (and possibly sphingomyelin) in membrane lipids. Thus, membrane lipids may form a storage site for choline, and their hydrolysis, with the subsequent release of choline, is highly regulated.



It is believed that the vitamin B12 requirement for choline synthesis contributes to the neurologic symptoms of vitamin B12 deficiency. The methyl groups for choline synthesis are donated by SAM, which is converted to S-adenosylhomocysteine in the reaction. Recall that formation of SAM through recycling of homocysteine requires both tetrahydrofolate and vitamin B12 (unless extraordinary amounts of methionine are available to bypass the B12-dependent methionine synthase step).

Evan Applebod was placed on Redux, which increased the secretion of serotonin. Serotonin has been implicated in many processes, including mood control and appetite regulation. When serotonin levels are low, increased appetite, or depression, or both can occur. Thus, drugs that can increase serotonin levels may be able to control appetite and depression. Redux was a second-generation drug developed from fenfluramine, a known appetite suppressant. When first used, fenfluramine could not be resolved between two distinct optical isomers (d versus I). The I-isomer induced sleepiness, so to counteract this effect fenfluramine was often given with phentermine, which elevated epinephrine levels to counteract the drowsiness (the combination of drugs was known as fen/phen). Once the two isomers of fenfluramine could be resolved, Redux, dexfenfluramine, was developed. Because levels of serotonin have been linked to mood, many antidepressant drugs were developed that affect serotonin levels. The first of these were the MAO inhibitors; a second class are the tricyclics, and the third class are known as the SSRI (selective serotonin reuptake inhibitors). The SSRI block reuptake of serotonin from the synapse, leading to an elevated response to serotonin. Redux acted both as an SSRI but also increased the secretion of serotonin, leading to elevated levels of this compound in the synapse. None of the other drugs that affect serotonin levels have this effect.

Histamine elicits a number of effects on different tissues. Histamine is the major mediator of the allergic response and when released from mast cells (a type of white blood cell found in tissues) leads to vasodilation and an increase in the permeability of blood vessel walls. This leads to the allergic symptoms of a runny nose and watering eyes. When histamine is released in the lungs, the airways constrict in an attempt to reduce the intake of the allergic material. The ultimate result of this, however, is bronchospasm, which can lead to difficulty in breathing. In the brain, histamine is an excitatory neurotransmitter. Antihistamines block histamine from binding to its receptor. In the tissues, this will counteract histamine's effect on vasodilation and blood vessel wall permeability, but in the brain the effect is to cause drowsiness. The new generation of "non-drowsy" antihistamines have been modified such that they cannot pass through the blood-brain barrier. Thus, the effects on the peripheral tissues are retained with no effect on CNS histamine response.



Fig. 48.8. Synthesis and inactivation of histamine; note the different pathways for brain and peripheral tissues. SAM = S-adenosylmethionine; SAH = S-adenosylhomocysteine.



**Fig. 48.9.** Acetylcholine synthesis and degradation.

Choline is a common component of the diet but also can be synthesized in the human as part of the pathway for the synthesis of phospholipids (see Chapter 33). The only route for choline synthesis is via the sequential addition of three methyl groups from SAM to the ethanolamine portion of phosphatidylethanolamine to form phosphatidylcholine. Phosphatidylcholine is subsequently hydrolyzed to release choline or phosphocholine. Conversion of phosphatidylethanolamine to phosphatidylcholine occurs in many tissues, including liver and brain. This conversion is B6- and B12-dependent.

The acetyl group used for acetylcholine synthesis is derived principally from glucose oxidation to pyruvate and decarboxylation of pyruvate to form acetyl

The supply of choline in the brain can become rate-limiting for acetylcholine synthesis, and supplementation of the diet with lecithin (phosphatidylcholine) has been used to increase brain acetylcholine in patients suffering from tardive dyskinesia (often persistent involuntary movements of the facial muscles and tongue). The neonate has a very high demand for choline, for both brain lipid synthesis (phosphatidylcholine and sphingomyelin) and acetylcholine biosynthesis. High levels of phosphatidylcholine in the maternal milk and a high activity of a high-affinity transport system through the blood-brain barrier for choline in the neonate help to maintain brain choline concentrations. The fetus also has a high demand for choline, and there is a highaffinity transport system for choline across the placenta. The choline is derived from maternal stores, maternal diet, and synthesis primarily in the maternal liver. Because choline synthesis is dependent on folate and vitamin B12, the high fetal demand may contribute to the increased maternal requirement for both vitamins during pregnancy. CoA via the pyruvate dehydrogenase reaction. This is because neuronal tissues have only a limited capacity to oxidize fatty acids to acetyl CoA so that glucose oxidation is the major source of acetyl groups. Pyruvate dehydrogenase is found only in mitochondria. The acetyl group is probably transported to the cytoplasm as part of citrate, which is then cleaved in the cytosol to form acetyl CoA and oxaloacetate.

#### **INACTIVATION OF ACETYLCHOLINE** 2.

Acetylcholine is inactivated by acetylcholinesterase, which is a serine esterase that forms a covalent bond with the acetyl group. The enzyme is inhibited by a wide range of compounds (pharmacologic drugs and neurotoxins) that form a covalent bond with this reactive serine group. Neurotoxins such as Sarin (the gas used in Japanese subways by a terrorist group) and the nerve gas in the movie "The Rock" work through this mechanism. Acetylcholine is the major neurotransmitter at the neuromuscular junctions; inability to inactivate this molecule leads to constant activation of the nerve-muscle synapses, a condition that leads to varying degrees of paralysis.

#### **Glutamate and GABA** E.

#### SYNTHESIS OF GLUTAMATE 1

Glutamate functions as an excitatory neurotransmitter within the central nervous system, leading to the depolarization of neurons. Within nerve terminals, glutamate is generally synthesized de novo from glucose rather than taken up from the blood because its plasma concentration is low and it does not readily cross the blood-brain barrier.

Glutamate is primarily synthesized from the TCA cycle intermediate  $\alpha$ -ketoglutarate (Fig. 48.10). This can occur via either of two routes. The first is via the enzyme glutamate dehydrogenase, which reduces  $\alpha$ -ketoglutarate to glutamate, thereby incorporating free ammonia into the carbon backbone. The ammonia pool is provided by amino acid /neurotransmitter degradation or by diffusion of ammonia across the blood-brain barrier. The second route is through transamination reactions in which an amino group is transferred from other amino acids to  $\alpha$ ketoglutarate to form glutamate. Glutamate also can be synthesized from glutamine, using glutaminase. The glutamine is derived from glial cells as described in section F.2.

Like other neurotransmitters, glutamate is stored in vesicles, and its release is  $Ca^{2+}$  -dependent. It is removed from the synaptic cleft by high-affinity uptake systems present in nerve terminals and glial cells.

#### 2. GABA

GABA ( $\gamma$ -aminobutyric acid) is the major inhibitory neurotransmitter in the central nervous system. Its functional significance is far-reaching, and altered GABA-ergic function plays a role in many neurologic and psychiatric disorders.

GABA is synthesized by the decarboxylation of glutamate (see Fig. 48.10) in a single step catalyzed by the enzyme glutamic acid decarboxylase (GAD). GABA is recycled in the central nervous system by a series of reactions called the GABA shunt, which conserves glutamate and GABA (see Fig. 48.10).

Much of the uptake of GABA occurs in glial cells. The GABA shunt in glial cells produces glutamate, which is converted to glutamine and transported out of the glial cells to neurons, where it is converted back to glutamate. Glutamine thus serves as a transporter of glutamate between cells in the CNS (see Chapter 42). Glial cells lack GAD and cannot synthesize GABA.

An inherited pyruvate dehydrogenase deficiency, a thiamine deficiency, or hypoxia deprives the brain of a source of acetyl CoA for acetylcholine synthesis, as well as a source of acetyl CoA for ATP generation from the TCA cycle.



The β-cells of the pancreas also express glutamate decarboxylase (GAD), and patients with type 1 diabetes express autoantigens against pancreatic GAD. Studies in a mouse model of type 1 diabetes have implicated GAD as a key autoantigen. If GAD expression is blocked in these mice by use of genetic tricks, diabetes does not develop. As usual, what occurs in the human condition is not as straightforward as it appears to be in animals, but there is a relationship between pancreatic GAD expression and the cause of type 1 diabetes in humans.

Tiagabine is a drug that inhibits the reuptake of GABA from the synapse, and it has been used to treat epilepsy as well as other convulsant disorders. Because GABA is an inhibitory neurotransmitter in the brain, its prolonged presence can block neurotransmission by other agents, thereby reducing the frequency of convulsions.



**Fig. 48.10.** Synthesis of glutamate and GABA and the GABA shunt. GDH = glutamate dehydrogenase; PLP = pyridoxal phosphate.

#### G. Other Amino Acid Neurotransmitters

#### 1. ASPARTATE

Aspartate, like glutamate, is an excitatory neurotransmitter, but it functions in far fewer pathways. It is synthesized from the TCA cycle intermediate oxaloacetate via transamination reactions. Like glutamate synthesis, aspartate synthesis uses oxaloacetate that must be replaced through anaplerotic reactions. Aspartate cannot pass through the blood-brain barrier.

#### 2. GLYCINE

Glycine is the major inhibitory neurotransmitter in the spinal cord. Most of the glycine in neurons is synthesized de novo within the nerve terminal from serine by the enzyme serine hydroxymethyltransferase, which requires folic acid. Serine, in turn, is synthesized from the intermediate 3-phosphoglycerate in the glycolytic pathway. The action of glycine is probably terminated via uptake by a high-affinity transporter.

#### 3. CONVERSION OF ARGININE TO NITRIC OXIDE

Nitric oxide (NO) is a biologic messenger in a variety of physiologic responses, including vasodilation, neurotransmission, and the ability of the immune system to

kill tumor cells and parasites. NO is synthesized from arginine in a reaction catalyzed by NO synthase (see Fig. 24.10).

NO synthase exists as tissue-specific forms of two families of enzymes. The form present in macrophages is responsible for the overproduction of NO, leading to its cytotoxic actions on parasites and tumor cells. The enzyme present in nervous tissue, vascular endothelium, platelets, and other tissues is responsible for the physiologic responses to NO such as vasodilation and neural transmission. In target cells, NO activates a soluble guanylate cyclase, which results in increased cellular levels of cGMP (3', 5' -cyclic GMP)(Fig. 48.11). In smooth muscle cells, cGMP, like cAMP, activates one or more protein kinases, which are responsible for the relaxation of smooth muscle and the subsequent dilation of vessels. NO stimulates penile erection by acting as a neurotransmitter, stimulating smooth muscle relaxation that permits the corpus cavernosum to fill with blood. Nitric oxide can readily cross cell membranes because it is a gas. As a result, its effect may not necessarily be limited to the neuron that synthesizes it (Fig. 48.12). There is ample evidence that NO may function as a retrograde messenger that can influence neurotransmitter release from the presynaptic terminal after diffusing from the postsynaptic neuron (where it is synthesized). There is also evidence supporting retrograde messenger roles for both arachidonic acid and carbon monoxide in the CNS.

## IV. METABOLIC ENCEPHALOPATHIES AND NEUROPATHIES

The brain has an absolute dependence on the blood for its supply of glucose and oxygen. It uses approximately 20% of the oxygen supply of the body. During the developmental period and during prolonged fasting, ketone bodies can be used as a fuel, but they cannot totally substitute for glucose. Glucose is converted to pyruvate in glycolysis, and the pyruvate is oxidized in the TCA cycle. Anaerobic glycolysis, with a yield of 2 ATPs/glucose, cannot sustain the ATP requirement of the brain, which can be provided only by the complete oxidation of glucose to  $CO_2$ , which yields approximately 32 ATPs /glucose. However, during periods of mild hypoglycemia or mild hypoxia, decreased neurotransmitter synthesis contributes as much, if not more, to the development of symptoms as does an absolute deficiency of ATP for energy needs.

## A. Hypoglycemic Encephalopathy

Hypoglycemia is sometimes encountered in medical conditions such as malignancies producing insulin, insulin-like growth factors, or chronic alcoholism. Early clinical signs in hypoglycemia reflect the appearance of physiologic protective mechanisms initiated by hypothalamic sensory nuclei such as sweating, palpitations, anxiety, and hunger. If unheeded, these symptoms give way to a more serious CNS disorder progressing through confusion and lethargy to seizures and eventually coma. Prolonged hypoglycemia can lead to irreversible brain damage.

During the progression of hypoglycemic encephalopathy, as blood glucose falls below 2.5 mM (45 mg/dL), the brain attempts to use internal substrates such as glutamate and TCA cycle intermediates as fuels. Because the pool size of these substrates is quite small, they are quickly depleted. If blood glucose levels continue to fall below 1 mM (18 mg/dL), ATP levels become depleted.

As the blood glucose drops from 2.5 to 2.0 mM (45 to 36 mg/dL, before EEG changes are observed), the symptoms appear to arise from decreased synthesis of neurotransmitters in particular regions of the brain rather than a global energy deficit. Figure 48.13 summarizes the relationship between the oxidation of glucose in glycolysis and the provision of precursors for the synthesis of neurotransmitters in different types of neurons.



Fig. 48.11. Action of nitric oxide (NO) in vasodilation. The synthesis of NO occurs in response to a stimulator binding to a receptor on some cells or to a nerve impulse in neurons. NO enters smooth muscle cells, stimulating guanylate cyclase to produce cGMP, which causes smooth muscle cell relaxation. When the smooth muscle cells relax, blood vessels dilate.



Fig. 48.12. NO as a retrograde messenger. Cell A releases a neurotransmitter, which stimulates cell C to produce NO. NO, being a gas, can diffuse back and regulate cell A's production and release of neurotransmitters. NO can also diffuse to cell B, and to stimulate cell B to produce a different neurotransmitter to elicit a response from cell D. NT = neurotransmitter.



**Fig. 48.13.** Glucose metabolism leading to the synthesis of the neurotransmitters glycine, aspartate, glutamate, and GABA. As blood glucose levels drop and brain glucose levels diminish, synthesis of these neurotransmitters may be compromised.

As hypoglycemia progresses below 1 mM (18 mg/dL), and high-energy phosphate levels are depleted, the EEG becomes isoelectric, and neuronal cell death ensues. As is the case in some other metabolic encephalopathies, cell death is not global in distribution; rather, certain brain structures, in particular hippocampal and cortical structures, are selectively vulnerable to hypoglycemic insult. Pathophysiologic mechanisms responsible for neuronal cell death in hypoglycemia include the involvement of glutamate excitotoxicity. Glutamate excitotoxicity occurs when the cellular energy reserves are depleted. The failure of the energy-dependent reuptake pumps results in a buildup of glutamate in the synaptic cleft and overstimulation of the postsynaptic glutamate receptors. The prolonged glutamate receptor activation leads to prolonged opening of the receptor ion channel and the influx of lethal amounts of Ca<sup>+2</sup> ion, which can activate cytotoxic intracellular pathways in the postsynaptic neuron.

## **B. Hypoxic Encephalopathy**

Experimental studies with human volunteers show that cerebral energy metabolism remains normal when mild to moderate hypoxia (partial pressure of oxygen, or  $PaO_2 = 25-40 \text{ mm Hg}$ ) results in severe cognitive dysfunction. The diminished cognitive function is believed to result from impaired neurotransmitter synthesis. In mild hypoxia, cerebral blood flow increases to maintain oxygen delivery to the brain. In addition, anaerobic glycolysis is accelerated, resulting in maintenance of ATP levels. This occurs, however, at the expense of an increase of lactate production and a fall of pH. Acute hypoxia (PaO<sub>2</sub>  $\leq$  20 mm Hg) generally results in a coma.

Hypoxia can result from insufficient oxygen reaching the blood (e.g., at high altitudes), severe anemia (e.g., iron deficiency), or a direct insult to the oxygen-utilizing capacity of the brain (e.g., cyanide poisoning). All forms of hypoxia result in diminished neurotransmitter synthesis. Inhibition of pyruvate dehydrogenase diminishes acetylcholine synthesis, which is acutely sensitive to hypoxia. Glutamate and GABA synthesis, which depend on a functioning TCA cycle, are decreased as a result of elevated NADH levels, which inhibit TCA cycle enzymes. NADH levels are increased when oxygen is unavailable to accept electrons from the electron transport chain and NADH cannot be converted back into NAD<sup>+</sup>. Even the synthesis of catecholamine neurotransmitters may be decreased because the hydroxylase reactions require  $O_2$ .

## C. The Relationship Between Glutamate Synthesis and the Anaplerotic Pathways of Pyruvate Carboxylase and Methylmalonyl CoA Mutase

Synthesis of glutamate removes  $\alpha$ -ketoglutarate from the TCA cycle, thereby decreasing the regeneration of oxaloacetate in the TCA cycle. Because oxaloacetate is necessary for the oxidation of acetyl CoA, oxaloacetate must be replaced by anaplerotic reactions. There are two major types of anaplerotic reactions: (1) pyruvate carboxylase and (2) the degradative pathway of the branched-chain amino acids, valine and isoleucine, which contribute succinyl CoA to the TCA cycle. This pathway uses B12 (but not folate) in the reaction catalyzed by methylmalonyl CoA mutase.

## V. LIPID SYNTHESIS IN THE BRAIN AND PERIPHERAL NERVOUS SYSTEM

A number of features of lipid synthesis and degradation in the nervous system distinguish it from most other tissues. The first is that the portion of the neuronal cell membrane involved in synaptic transmission has a unique role and a unique composition. At the presynaptic terminal, the lipid composition is rapidly changing as storage vesicles containing the neurotransmitter fuse with the cell membrane and release their contents. Portions of the membrane are also lost as endocytotic vesicles. On the postsynaptic terminal, the membrane contains the receptors for the neurotransmitter as well as a high concentration of membrane signaling components, such as phosphatidylinositol. A second important feature of brain lipid metabolism is that the blood-brain barrier restricts the entry of nonessential fatty acids such as palmitate, which are released from adipose tissue or present in the diet. Conversely, essential fatty acids are taken up by the brain. Because of these considerations, the brain is constantly synthesizing those lipids (cholesterol, fatty acids, glycosphingolipids, and phospholipids), which it needs for various neurologic functions. Neuronal signaling also requires that non-neuronal glial cells synthesize myelin, a multilayered membrane that surrounds the axons of many neurons. Myelin is lipid rich and has a different lipid composition than the neuronal membranes. The white matter in the brain contains significantly more myelin than the gray matter; it is the presence of myelin sheaths that is responsible for the characteristic color differences that exist between the two types of brain tissue.

#### A. Brain Lipid Synthesis and Oxidation

Because the blood-brain barrier significantly inhibits the entry of certain fatty acids and lipids into the CNS, virtually all lipids found there must be synthesized within the CNS. The exceptions are the essential fatty acids (linoleic and linolenic acid), which do enter the brain, where they are elongated or further desaturated. The uptake of fatty acids into the CNS is insufficient to meet the energy demands of the CNS; hence the requirement for aerobic glucose metabolism. Thus, cholesterol, glycerol, and sphingolipids, glycosphingolipids, and cerebrosides are all synthesized using pathways previously discussed in this text. Of particular note is that very-long-chain fatty acids are synthesized in the brain, where they play a major role in myelin formation. Oxidation and turnover of brain lipids occurs as described previously in the text (see Chapter 23). Peroxisomal fatty acid oxidation is important in the brain because the brain contains very-long-chain fatty acids and phytanic acid (from the diet), both of which are oxidized in the peroxisomes by  $\alpha$ -oxidation. Thus, disorders that affect peroxisome biogenesis (such as Refsum's disease) severely affect brain cells because of the inability to metabolize both branched-chain and very-long-chain fatty acids. If there is a disorder in which the degradation of glycosphingolipids or mucopolysaccharides is reduced, lysosomes in brain cells will become engorged with partially digested glycolipids, leading to varying degrees of neurologic dysfunction.

#### **B. Myelin Synthesis**

A rapid rate of nerve conduction in the peripheral and central motor nerves depends on the formation of myelin, a multilayered lipid and protein structure that is formed from the plasma membrane of glial cells. In the peripheral nervous system, the Schwann cell is responsible for myelinating one portion of an axon of one nerve cell. The Schwann cell does this by wrapping itself around the axon multiple times such that a multilayered sheath of membrane surrounds the axon. In the central nervous system, the oligodendrocyte is responsible for myelination. Unlike the Schwann cell, oligodendrocytes can myelinate portions of numerous axons (up to 40), and do so by extending a thin process that wraps around the axon multiple times Thus, CNS axons are only surrounded by the entire Schwann cell. A generalized view of myelination is depicted in Figure 48.14. To maintain the myelin structure, the oligodendrocyte synthesizes 4 times its own weight in lipids per day.

#### 1. MYELIN LIPIDS

As the plasma membrane of the glial cell is converted into myelin, the lipid composition of the brain changes (Table 48.2). The lipid-to-protein ratio is greatly increased, as is the content of sphingolipids. The myelin is a tightly packed structure,



**Fig. 48.14.** A composite diagram indicating a Schwann cell that has wrapped around a portion of an axon, forming the myelin sheath. The expansion represents a portion of the myelin sheath. CNS myelin is shown, although it is similar to PNS myelin except that Po would take the place of PLP (proteolipid protein). Recall that there are multiple layers of membrane surrounding the axon; PLP protrudes into the extracellular space and aids in compaction of the membranes through hydrophobic interactions. MBPs help to stabilize the structure from within the membrane.

Substance <sup>a</sup>	Myelin	White Matter	Gray Matter
Protein	30.0	39.0	55.3
Lipid	70.0	54.9	32.7
Cholesterol	27.7	27.5	22.0
Cerebroside	22.7	19.8	5.4
Sulfatide	3.8	5.4	1.7
Total galactolipid	27.5	26.4	7.3
Ethanolamine phosphatides	15.6	14.9	22.7
Phosphatidylcholine	11.2	12.8	26.7
Sphingomyelin	7.9	7.7	6.9
Phosphatidylserine	4.8	7.9	8.7
Phosphatidylinositol	0.6	0.9	2.7
Plasmalogens	12.3	11.2	8.8
Total phospholipids	43.1	45.9	69.5

Table 48.2. Protein	and Lipid Composition	of CNS Myelin and Human Brain
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<sup>a</sup>Protein and lipid figures in percent dry weight; all others in percent total lipid weight

Data from W. Norton, in Siegel GJ, Albers RW, Agranoff BW, Katzman R, eds. Basic Neurochemistry, 3rd Ed. Boston: Little Brown & Co, 1981:77.

and there are significant hydrophobic interactions between the lipids and proteins to allow this to occur. Cerebrosides constitute approximately 16% of total myelin lipid and are almost completely absent from other cell-type membrane lipids. The predominant cerebroside, galactosylcerebroside, has a single sugar attached to the hydroxyl group of the sphingosine. In contrast, sphingomyelin, which one might guess is the predominant lipid of myelin, is present in roughly the same low concentration in all membranes. Galactocerebrosides pack more tightly together than phosphatidylcholine; the sugar, although polar, carries no positively charged amino group or negatively charged phosphate. The brain synthesizes very-long-chain fatty acids (greater than 20 carbons long); these long uncharged side chains develop strong hydrophobic associations, allowing a close packing of the myelin sheath. The high cholesterol content of the membrane also contributes to the tight packing, although the myelin proteins are also required to complete the tightness of the packing process.

#### 2. MYELIN STRUCTURAL PROTEINS

The layers of myelin are held together by protein/lipid and protein/protein interactions, and any disruption can lead to demyelination of the membrane (see Biochemical Comments). Although numerous proteins are found in both the CNS and PNS, only the major proteins are discussed here. The major proteins in the CNS and PNS are different. In the CNS, two proteins constitute between 60 and 80% of the total proteins—proteolipid protein (PLP) and myelin basic proteins (MBP). The PLP is a very hydrophobic protein that forms large aggregates in aqueous solution and is relatively resistant to proteolysis. Its molecular weight, based on sequence analysis, is 30,000 Daltons. PLP is highly conserved in sequence amongst species. Its role is thought to be one of promoting the formation and stabilization of the multilayered myelin structure.

The MBPs are a family of proteins. Unlike PLP, MBPs are easily extracted from the membrane and are soluble in aqueous solution. The major MBP has no tertiary structure and has a molecular weight of 15,000 Daltons. MBP is located on the cytoplasmic face of myelin membranes. Antibodies directed against MBPs elicit experimental allergic encephalomyelitis (EAE), which has become a model system for understanding multiple sclerosis, a demyelinating disease. A model of how PLP and MBPs aid in stabilizing myelin is shown in Figure 48.14.

In the PNS, the major myelin protein is Po, a glycoprotein that accounts for greater than 50% of the PNS myelin protein content. The molecular weight of Po is 30,000, the same as PLP. Po is thought to play a similar structural role in maintaining

myelin structure, as does PLP in the CNS. Myelin basic proteins are also found in the PNS, with some similarities and differences to the MBPs found in the CNS. The major PNS-specific MBP has been designated P2.

#### **CLINICAL COMMENTS**

Catecholamines affect nearly every tissue and organ in the body. Their integrated release from nerve endings of the sympathetic (adrenergic) nervous system plays a critical role in the reflex responses we make to sudden changes in our internal and external environment. For example, under stress, catecholamines appropriately increase heart rate, blood pressure, myocardial (heart muscle) contractility, and conduction velocity of the heart.

Episodic, inappropriate secretion of catecholamines in pharmacologic amounts, such as occurs in patients with pheochromocytomas, like **Katie Colamin**, causes an often alarming array of symptoms and signs of a hyperadrenergic state.

Most of the signs and symptoms related to catecholamine excess can be masked by phenoxybenzamine, a long-acting  $\alpha_1$ - and  $\alpha_2$ -adrenergic receptor antagonist, combined with a  $\beta_1$ - and  $\beta_2$ -adrenergic receptor blocker such as propranolol. Pharmacologic therapy alone is reserved for patients with inoperable pheochromocytomas (e.g., patients with malignant tumors with metastases and patients with severe heart disease). Because of the sudden, unpredictable, and sometimes lifethreatening discharges of large amounts of catecholamines from these tumors, definitive therapy involves surgical resection of the neoplasms(s) after appropriate preoperative preparation of the patient with the agents mentioned above. Katie's tumor was resected without intraoperative or postoperative complications. After surgery, she remained free of symptoms, and her blood pressure decreased to normal levels.

**Evan Applebod**, after stopping Redux, was placed on Prozac, an antidepressant that acts as a specific serotonin reuptake inhibitor (SSRI) but does not lead to increased synthesis or secretion of serotonin, as did dexfenfluramine in Redux. Thus, the mechanism of action of these two drugs is different, even if the end result (elevated levels of serotonin) is the same. Unfortunately, Prozac did not work as well for Mr. Applebod as did Redux, and he regained his 100 pounds within a year after switching medications. Redux was withdrawn from the market by its manufacturer because of reports of heart valve abnormalities in a small percentage of patients who had taken either phen/fen or Redux. Since then, the FDA has banned the use of Redux for weight loss because of the undesirable side effects. Other treatments, such as orlistat, a partial inhibitor of dietary fatty acid absorption from the gastrointestinal tract, are being tried in an effort to effect weight loss safely in patients like **Evan Applebod**.

#### **BIOCHEMICAL COMMENTS**

The importance of myelin in nerve transmission is underscored by the wide variety of demyelinating diseases, all of which lead to neurologic symptoms. The best known disease in this class is multiple sclerosis (MS). MS can be a progressive disease of the CNS in which demyelination of CNS neurons is the key anatomic and pathologic finding. The cause of MS has yet to be determined, although it is believed that an event occurs that triggers the formation of autoimmune antibodies directed against components of the nervous system. This

event could be a bacterial or viral infection that stimulates the immune system to fight off the invaders. Unfortunately, this stimulus may also trigger the autoimmune response that leads to the antibody-mediated demyelinating process. The unusual geographic distribution of MS is of interest. Patients are concentrated in northern and southern latitudes, yet its incidence is almost nil at the equator. Clinical presentation of MS varies widely. Most commonly it is a mild disease that has few or no obvious clinical manifestations. At the other end of the spectrum is a rapidly progressive and fatal disease. The most well-known presentation is the relapsing-remitting type. In this type, early in the course of the disease, the natural history is one of exacerbations, followed by remission. Eventually the CNS cannot repair the damage that has accumulated through the years, and remissions occur less and less frequently. Available treatments for MS target the relapsing-remitting type of disease.

The primary injury to the CNS in MS is the loss of myelin in the white matter, which interferes with nerve conduction along the demyelinated area (the insulator is lost). The CNS compensates by stimulating the oligodendrocyte to remyelinate the damaged axon, and when this occurs, remission is achieved. Often remyelination leads to a slowing in conduction velocity because of a reduced myelin thickness (speed is proportional to myelin thickness) or a shortening of the internodal distances (the action potential has to be propagated more times). Eventually, when it becomes too difficult to remyelinate large areas of the CNS, the neuron adapts by upregulating and redistributing along its membrane ion pumps, to allow nerve conductance along demyelinated axons. Eventually this adaptation also fails, and the disease progresses.

Treatment of MS is now based on blocking the action of the immune system. Because antibodies directed against cellular components appear to be responsible for the progression of the disease (regardless of how the autoantibodies were first generated), agents that interfere with immune responses have had various levels of success in keeping patients in remission for extended periods.

Other demyelinating diseases also exist, and their cause is much more straightforward. These are relatively rare disorders. In all of these diseases, there is no fully effective treatment for the patient. Inherited mutations in Po (the major PNS myelin protein) leads to a version of Charcot-Marie-Tooth polyneuropathy syndrome. The inheritance pattern for this disease is autosomal dominant, indicating that the expression of one mutated allele will lead to expression of the disease. Mutations in PLP (the major myelin protein in the CNS) lead to Pelizaeus-Merzbacher disease and X-linked spastic paraplegia type 2 disease. These diseases display a wide range of phenotypes, from a lack of motor development and early death (most severe) to mild gait disturbances. The phenotype displayed depends on the precise location of the mutation within the protein. An altered function of either Po or PLP leads to demyelination and its subsequent clinical manifestations.

#### **Suggested Readings**

- Herndon RM. Multiple Sclerosis: Immunology, Pathology, and Pathophysiology. New York: Demos Medical Publishing, 2003.
- Lupski JR, Garcia CA. Charcot-Marie-Tooth peripheral neuropathies and related disorders. In: Scriver CR, Beaudet AL, Valle D, Sly WS, et al eds. The Metabolic and Molecular Bases of Inherited Disease, vol II, 8th Ed. New York: McGraw-Hill, 2001:5759–5788.
- Siegel G, Agranoff B, Albers RW, Molinoff P, eds. Basic Neurochemistry, 4<sup>th</sup> Ed. New York: Raven Press, 1989.

Webster RA, ed. Neurotransmitters, Drugs and Brain Function. London: John Wiley and Sons Ltd, 2001.

# **REVIEW QUESTIONS-CHAPTER 48**

- 1. A patient with a tumor of the adrenal medulla experienced palpitations, excessive sweating, and hypertensive headaches. His urine contained increased amounts of vanillylmandelic acid. His symptoms are probably caused by an overproduction of which of the following?
  - (A) Acetylcholine
  - (B) Norepinephrine and epinephrine
  - (C) DOPA and serotonin
  - (D) Histamine
  - (E) Melatonin
- 2. The two lipids found in highest concentration in myelin are which of the following?
  - (A) Cholesterol and cerebrosides such as galactosylceramide
  - (B) Cholesterol and phosphatidylcholine
  - (C) Galactosylceramide sulfatide and sphingomyelin
  - (D) Plasmalogens and sphingomyelin
  - (E) Triacylglycerols and lecithin
- 3. Myelin basic protein can best be described by which of the following?
  - (A) It is synthesized in Schwann cells, but not in oligodendrocytes.
  - (B) It is a transmembrane protein found only in peripheral myelin.
  - (C) It attaches the two extracellular leaflets together in central myelin.
  - (D) It contains basic amino acid residues that bind the negatively charged extracellular sides of the myelin membrane together.
  - (E) It contains lysine and arginine residues that bind the negatively charged intracellular sides of the myelin membrane together.
- 4. A patient presented with dysmorphia and cerebellar degeneration. Analysis of his blood indicated elevated levels of phytanic acid and very-long-chain fatty acids, but no elevation of palmitate. His symptoms are consistent with a defect in an enzyme involved in which of the following?
  - (A)  $\alpha$ -Oxidation
  - (B) Mitochondrial β-oxidation
  - (C) Transport of enzymes into lysosomes
  - (D) Degradation of mucopolysaccharides
  - (E) Elongation of fatty acids
- 5. One of the presenting symptoms of vitamin B6 deficiency is dementia. This may result from an inability to synthesize serotonin, norepinephrine, histamine, and GABA from their respective amino acid precursors. This is because B6 is required for which type of reaction?
  - (A) Hydroxylation
  - (B) Transamination
  - (C) Deamination
  - (D) Decarboxylation
  - (E) Oxidation