3.2 Choline and Its Products Acetylcholine and Phosphatidylcholine

AU1

Choline

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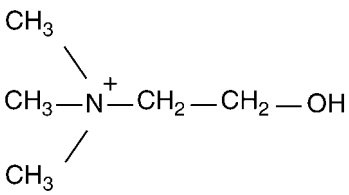
AU2 **Abstract:** Choline, a quaternary amine obtained largely from the diet but also synthesized in the brain and, especially, liver, is an essential precursor of the neurotransmitter acetylcholine (ACh) and of the major membrane constituent phosphatidylcholine (PC). Plasma choline concentrations can vary over a fivefold range depending on the choline contents of the foods being digested. Since choline readily crosses the blood–brain barrier (BBB) through an unsaturated facilitated-diffusion system, these plasma changes can produce parallel changes in brain choline levels. In addition, since the enzymes that convert choline to ACh [choline acetyltransferase (ChAT)] and PC's precursor phosphocholine [choline kinase (CK)] are also poorly saturated with their choline substrate, increases in plasma choline can enhance the formation of ACh and phosphocholine, and the release of ACh. The subsequent conversion of phosphocholine to PC is increased if PC's other circulating precursors (uridine and omega-3 fatty acids) are provided. This leads to an increase in the levels of synaptic membrane within the brain. Choline is principally metabolized in the liver to betaine, which provides a source of methyl groups for the regeneration of methionine and S-adenosylmethionine.

1 Introduction

Choline (2-hydroxyethyl-trimethyl-ammonium), a simple but unusual compound, consists of a 2-carbon chain in which one carbon is attached to a hydroxyl group and the other to an amine nitrogen (◉ *Figure 3.2-1*). The particularly unusual quality of choline is that this amine nitrogen bonds with a total of four

◘ Figure 3.2-1
Structure of choline



hydrogen or carbon atoms instead of with the usual three, and thus carries a partial positive charge. Though choline is not an amino acid, it shares with that family of compounds the property of being present in cells both in free form and—like the amino acids in proteins—within subunits (e.g., PC) of a macromolecule (biologic membranes). Moreover, like tyrosine, tryptophan (Cansev and Wurtman, 2006), and histidine, choline is the precursor of a neurotransmitter (ACh), and also like tyrosine and histidine, choline must be obtained from both endogenous synthesis and dietary sources.

This chapter describes the choline in the blood and, particularly, in the brain, —its sources; its uses to make membrane phospholipids and ACh; and its other biologic effects.

2 Choline in the Blood

Choline is a normal constituent of the plasma (◉ *Table 3.2-1*), present as the free base (Cohen and Wurtman, 1975; Hirsch et al., 1978; Savendahl et al., 1997); as a constituent of phospholipids [including PC; sphingomyelin (SM); lyso-PC; choline-containing plasmalogens; and the platelet-activating factor (PAF)] and as PC's water-soluble metabolites [principally phosphocholine and glycerophosphocholine (GPC) (Hirsch et al., 1978; Klein et al., 1993; Ilcol et al., 2005a)]. Free choline is also found in other biological fluids (◉ *Table 3.2-1*). In blood, choline is concentrated within erythrocytes (10–150 μM;

Table 3.2-1
Free choline concentrations in human body fluids

Body fluids	Free choline (μM)	References
Plasma	7.0–13.0	Holm et al. (2003)
Serum	9.0–13.3	Holm et al. (2003); Ilcol et al. (2002b, 2005a)
Urine	15.5 ± 8.8	Buchman et al. (1999)
Cerebrospinal fluid	0.7–2.5	Flentge et al. (1984); Ikeda et al. (1990); Toghi et al. (1996)
Amniotic fluid	22.8–24.5	Ilcol et al. (2002e)
Colostrum	132 ± 21	Ilcol et al. (2005a)
Breast milk	110–300	Holmes et al. (2000); Ilcol et al. (2005a)
Semen	17,000–24,000	Takatori et al. (1984); Manabe et al. (1991)
Peritoneal dialysate	14–28	Hjelle et al. (1993); Ilcol et al. (2002b)

Note: Values are given as the range of the means from the cited references, or as the mean ± SEM

Jope et al., 1980; Stoll et al., 1991) through the action of an uptake molecule that is unsaturated (K_m = 5–10 μM; Riley et al., 1997) at normal plasma choline concentrations.

2.1 Sources of Plasma Choline

Plasma choline derives from three sources—dietary choline, consumed as the free base or as a constituent of phospholipid molecules; endogenous synthesis, principally in liver; and liberation from its reservoir within the membrane phosphatides of all mammalian cells.

2.1.1 Dietary Choline

Choline is present within many foods (*Table 3.2-2*), principally as the free molecule or as phosphatides, and its plasma levels can rapidly increase severalfold after ingestion of choline-rich foods (Hirsch et al., 1978). For example, the consumption of a 5-egg omelet (containing about 1.3 g of choline) by humans increased these levels from 9.8 μM to 36.6 μM within 4 h (Hirsch et al., 1978). Prolonged fasting reduced human plasma choline levels from 9.5 μM to 7.8 μM after 7 days (Savendahl et al., 1997). Similarly, removal of all choline-containing foods from the diet for 17–19 days gradually lowered plasma choline, from 10.6 μM to 8.4 μM in humans (Zeisel et al., 1991) and from 12.1 μM to 6.3 μM in rats (Klein et al., 1998), indicating that plasma choline can be partially but not fully sustained by release from endogenous stores.

Dietary PC is deacylated within the gut to form lyso-PC. About half of this product is further degraded to free choline within the gut or liver. The remainder is reacylated to regenerate PC (Houtsmuller, 1979), which is then absorbed into the lymphatic circulation (Fox et al., 1979) and eventually enters the blood stream.

Much of the dietary choline that reaches the liver through the portal circulation is destroyed by oxidation to betaine, as described later (*Figure 3.2-2*), ultimately providing methyl groups that can be used to regenerate S-adenosylmethionine (SAM) from homocysteine. The rest of the choline in portal venous blood passes into the systemic circulation (Fox et al., 1979; Houtsmuller, 1979).

In 1998, the Food and Nutrition Board (FNB) of the US Institute of Medicine established a dietary reference intake (DRI) for choline. Since the FNB did not believe that existing scientific evidence allowed calculation of a recommended daily allowance (RDA) for choline, it instead set an adequate (daily) intake level (AI), and an upper (daily) limit (UL) that should not be exceeded (*Table 3.2-3*). The main criteria for determining the AI and UL were, respectively, the amount of choline needed to prevent liver damage,

Table 3.2-2
Choline contents of common foods

A. High						
Food	Choline	GPC	PCho	PC	SM	Total Choline
Egg yolk	1	1	1	634	45	682
Beef liver	61	83	11	245	24	424
Chicken liver	69	5	6	213	14	307
Cereals, ready to eat or wheat germ	69	33	4	44	0	150
Pork or bacon	12	18	3	86	10	129
Cake, chocolate, without frosting	5	61	1	58	2	127
Coffee, instant, decaffeinated	93	8	0	0	0	101
Cauliflower	24.5	0.7	1.8	12.1	0	39.1
B. Low						
Food	Choline	GPC	PCho	PC	SM	Total Choline
Olive oil	0	0.3	0	0	0	0.3
Kale	0.1	0	0	0.3	0	0.4
Iced tea	0.4	0	0	0	0	0.4
Egg white	0.2	0.6	0	0.3	0	1.1
Apple juice	0.7	0.7	0	0.4	0	1.8
Coffee, brewed from grounds	1.9	0.7	0	0.4	0	2.6

Note: Data are given as mg choline moiety/100 g of food. Foods are grouped based on having relatively high or low choline contents. Data from USDA Database for the Choline Content of Common Foods, 2004
GPC, glycerophosphocholine; PCho, phosphocholine; PC, phosphatidylcholine; SM, sphingomyelin

and the choline intake associated with choline's most sensitive adverse effect, i.e., hypotension (see Dietary Reference Intakes, Institute of Medicine, National Academy of Sciences USA, 1998). Subsequent studies have shown that the enzymes (described later) that synthesize and metabolize choline can be affected by common genetic polymorphisms, which cause important person-to-person variations in dietary choline needs (da Costa et al., 2006). For further details about dietary reference intakes and the choline contents of various foods, the reader is referred to the official websites of the Institute of Medicine (http://www.nap.edu/catalog/6015.html#toc) and the USDA (http://www.nal.usda.gov/fnic/foodcomp/Data/Choline/Choline.html).

2.1.2 Endogenous Choline Synthesis

Endogenous choline is produced, principally in liver (Bremer and Greenberg, 1960) but also to a small extent within brain (Blusztajn et al., 1979; Crews et al., 1980), by the sequential addition of three methyl groups to the amine nitrogen of phosphatidylethanolamine (PE); this forms PC, which can then be broken down to liberate the choline (*Figure 3.2-3*). The methylation reactions are catalyzed by two enzymes, phosphatidylethanolamine-*N*-methyltransferase (PEMT1; EC: 2.1.1.17), which converts PE to its monomethyl derivative, and phosphatidyl-*N*-methylethanolamine-*N*-methyltransferase (PEMT2; EC: 2.1.1.71), which adds the second and third methyl groups (A single enzyme may catalyze all three methylations in liver). Both enzymes use SAM as the methyl donor (Bremer and Greenberg, 1960; Hirata et al., 1978). Their K_ms for SAM are 2–4 $\times$ 10^{-6} M and 20–110 $\times$ 10^{-6} M, respectively (Crews et al., 1980; Blusztajn et al., 1982; Hitzemann, 1982; Percy et al., 1982), whereas brain SAM concentrations are 10–17 μg/g wet weight [50–85 μM assuming about 50% of the brain mass is aqueous (Wurtman and Rose, 1970; Ordonez and Wurtman, 1974)]. Hence, PEMT1 is probably fully saturated with SAM whereas PEMT2 is not.

Figure 3.2-2

Metabolism of choline to betaine, methionine and, S-adenosylmethionine (SAM). THF, Tetrahydrofolate; 5,10-MTHF, 5,10-methylene-tetrahydrofolate; 5-methyl-THF, 5-methyl-tetrahydrofolate; B12, Vitamin B12; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine

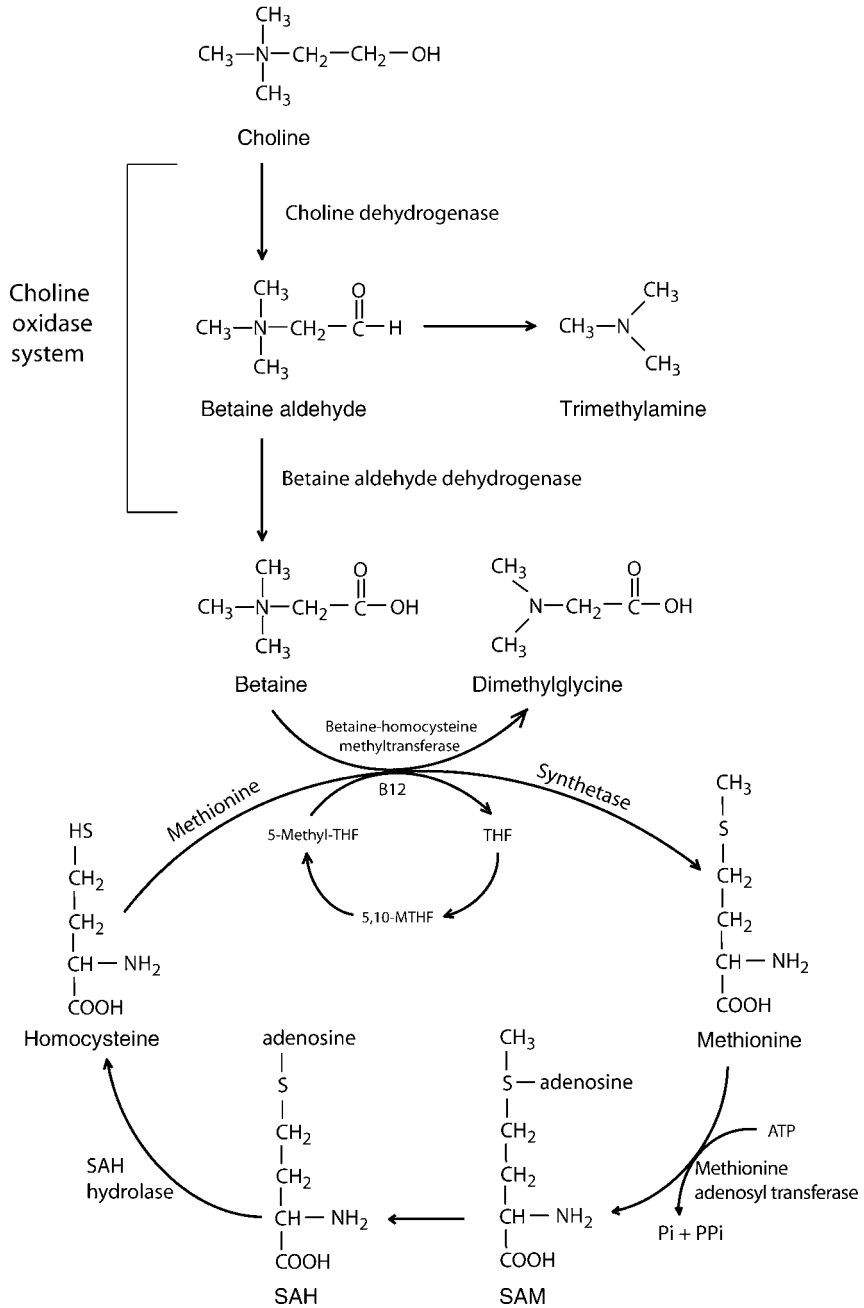


Table 3.2-3
Proposed adequate (i.e., minimum) daily choline intake, and upper limit (which should not be exceeded)

A. Adequate Intakes (AI)		
Age	**Gender**	**AI (mg/day)**
0–6 months	Both	125
7–12 months	Both	150
1–3 years	Both	200
4–8 years	Both	250
9–13 years	Male	375
14–18 years	Male	550
9–13 years	Female	375
14–18 years	Female	400
Above 19 years	Male	550
Above 19 years	Female	425
Pregnancy		450
Lactation		550
B. Upper Allowable Intakes (UL, Upper Limit)		
Life stage	**Age**	**UL (g/day)**
Infancy	0–12 months	N/A[a]
Childhood	1–8 years	1
	9–13 years	2
Adolescence	14–18 years	3
Pregnancy	14–18 years	3
	19 years and older	3.5
Lactation	14–18 years	3
	19 years and older	3.5

[a]Not possible to establish; sources of intake should only be mother's milk and infant formulas

The gene for human PEMT2 has been localized to chromosome 17p11.2 (Walkey et al., 1999), and cDNA for PEMT2 has been cloned from rat liver and expressed in COS-1 cells (Cui et al., 1993). PEMT activity was identified in membranous fractions from homogenates of rat and bovine brain (Blusztajn et al., 1979; Mozzi and Porcellati, 1979; Crews et al., 1980); highest specific activies were present in synaptosomes (Blusztajn et al., 1979; Crews et al., 1980) suggesting that nerve terminals are able to synthesize choline. In the course of these transmethylations, the phosphatide intermediates "flip" from the membrane's cytoplasmic side, where most of the less-polar PE and phosphatidylserine (PS) are found, to the more polar external leaf (Hirata and Axelrod, 1978). PE itself can be formed in liver, kidney, or brain from free ethanolamine, via the CDP-ethanolamine cycle (or "Kennedy Cycle"; ❯ *Figure 3.2-4*) described later, or from the decarboxylation of PS (Kennedy, 1956; Borkenhagen et al., 1961). PS is produced, in nerve terminals (Holbrook and Wurtman, 1988) and elsewhere, by the process of "base-exchange," in which a serine molecule substitutes for the ethanolamine in PE or the choline in PC.

Free choline is liberated from newly synthesized PC and from PC molecules formed from preexisting choline, by a family of enzymes, the phospholipases (❯ *Figure 3.2-5*). Phospholipase D (PLD) acts directly on the choline/phosphate bond of PC to generate choline and phosphatidic acid (❯ *Figure 3.2-5a*). Phospholipase A2 (PLA2) acts on the bond connecting a fatty acid to the hydroxyl group on PC's 2-carbon to yield that fatty acid [often arachidonic acid (AA) or docosahexaenoic acid (DHA)] and lyso-PC (❯ *Figure 3.2-5b*). This lyso-PC can then be further metabolized to choline, either directly, through the action of a phosphodiesterase, or first to GPC, by phospholipase A1 (PLA1), and then to choline by a

Figure 3.2-3

Sequential methylation of phosphatidylethanolamine (PE) to phosphatidylcholine (PC). PEMT-I, Phosphatidylethanolamine-*N*-methyltransferase; PEMT-II, phosphatidyl-*N*-methylethanolamine-*N*-methyltransferase; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine

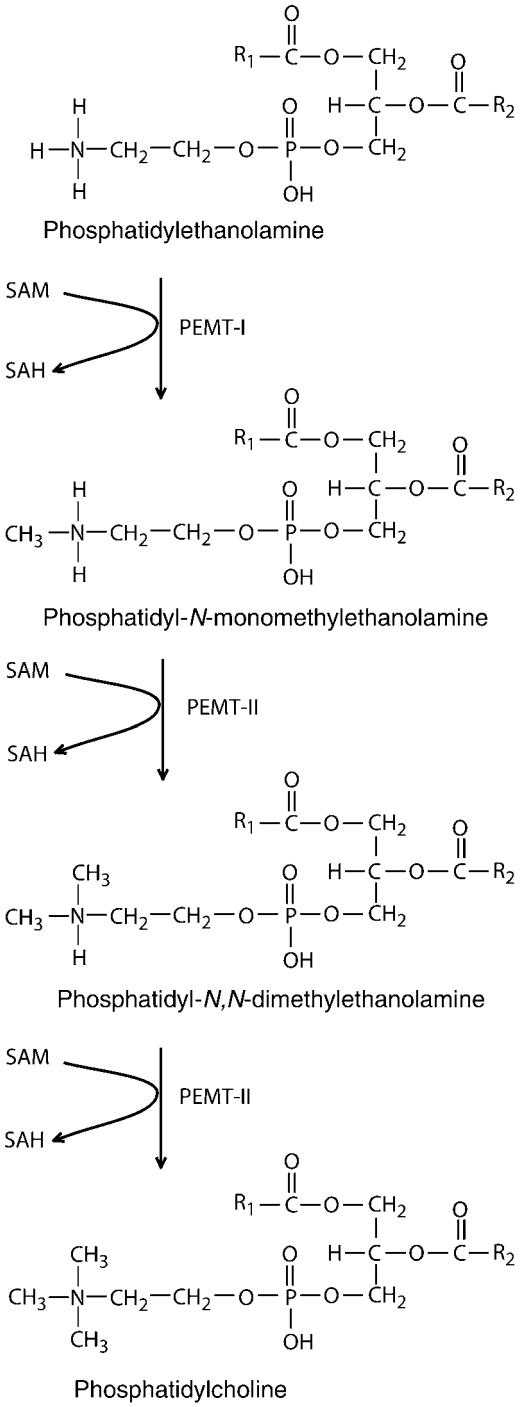
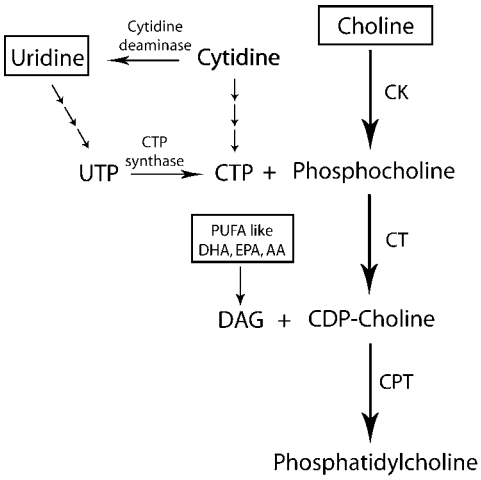


Figure 3.2-4

Biosynthesis of phosphatidylcholine (PC) from preexisting choline through the CDP-choline cycle ("Kennedy cycle"). Synthesis of PC is shown here. Synthesis of phosphatidylethanolamine (PE) is similar except that it uses ethanolamine instead of choline. Boxes indicate compounds most or all of which must be taken up into the brain from the circulation. Uridine is the principal circulating precursor of the CTP (cytidine-5′-triphosphate) needed for PC and PE synthesis through the Kennedy pathway, and exogenous cytidine is rapidly deaminated to uridine in humans (Wurtman et al., 2000). CK, Choline Kinase; CT: CTP: phosphocholine cytidylyltransferase; CPT: CDP-choline, 1,2-diacylglycerol cholinephosphotransferase; PUFA, Polyunsaturated fatty acid; DHA, Docosahexaenoic acid; EPA, Eicosapentaenoic acid; AA, Arachidonic acid



phosphatase. Phospholipase C (PLC) acts on the bond connecting the phosphate and the hydroxyl group on PC's 3-carbon to yield diacylglycerol (DAG) and phosphocholine; the phosphocholine can then be metabolized to free choline through the action of a phosphatase (*Figure 3.2-5c*).

It is estimated that, on average, about 15% of the free choline that enters the human blood stream derives from endogenous synthesis, the rest coming principally from dietary sources (Zeisel, 1981). Acute or chronic liver disease or deficiencies in methionine, folic acid, or vitamin B12 intake could thus lower plasma choline levels by impairing hepatic PC synthesis (*Table 3.2-4*).

2.1.3 Choline-Containing Membrane Phospholipids

Cellular membranes contain most of the choline in the body, principally in the form of the phosphatide PC, but also as PC's products SM (*Figure 3.2-6*) and lyso-PC (*Figure 3.2-5b*), or as less-abundant choline-containing phospholipids like the PAF (1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine). Membranes also contain the phosphatides PS, PE, and phosphatidylinositol (PI), as well as specific proteins, cholesterol, and various minor lipids. The quantities of choline present in brain as PC (2–2.5 mmoles/g) or as SM (0.25 mmoles/g) are orders of magnitude greater than those of free choline (30–60 μM). The proportion of any membrane's phospholipids represented by PC can vary depending on the species and age of the animal, the particular brain region or cell type being studied, and the membrane's function within the cell (e.g., nuclear membrane and plasma membrane) (Suzuki, 1981). In the gray matter of human brain, PC constitutes 42% of total phospholipids and SM 10%; in white matter, the proportions of PC and SM are 33% and 15%, respectively (Suzuki, 1981).

Moreover, "PC" is highly heterogeneous, actually representing a family of compounds with differing fatty acid compositions (*Figure 3.2-3*; Lee and Hajra, 1991) and, consequently, differing chemical and

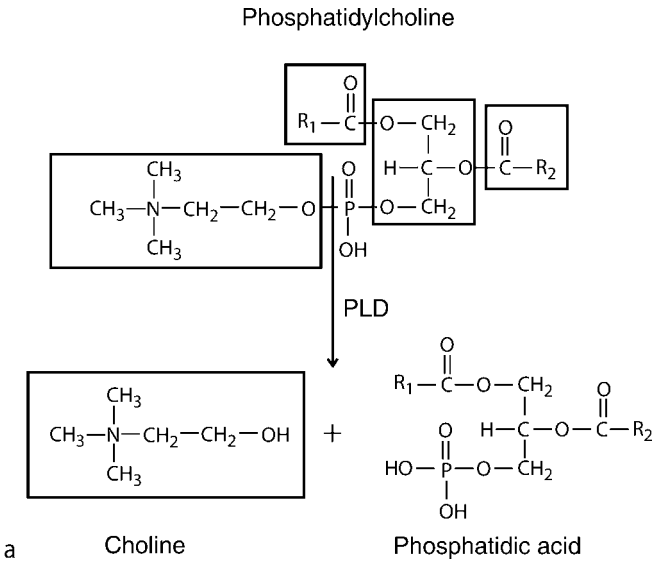
physical properties. The fatty acid in the C-1 position of PC tends most often to be saturated, e.g., stearic or palmitic acid, whereas that in position C-2 is more likely to be monounsaturated (oleic acid) or polyunsaturated [e.g., the omega-3 fatty acids (DHA; 22:6) and eicosapentenoic acid (EPA; 20:5) or the omega-6 fatty acid (AA; 20:4)]. Newly synthesized phosphatide molecules contain relatively larger quantities of polyunsaturated fatty acids (PUFA) than the phosphatide molecules present at steady state (Tacconi and Wurtman, 1985). This reflects either faster turnover of PUFA-containing phosphatides, or their rapid deacylation followed by reacylation with more saturated fatty acid species (Houtsmuller, 1979), or both. Membranes of retinal and brain cells are especially rich in PUFA, particularly DHA [which comprises about 20% of the total fatty acids in retinal phospholipids (Futterman and Andrews, 1964; Martinez, 1992) and about 7% of those in brain phospholipids (Martinez, 1992), respectively]. As described later, administration of supplemental DHA accelerates PC synthesis and increases brain levels of PC and other phosphatides (Wurtman et al., 2006).

2.2 Fates of Circulating Choline

Plasma choline can be taken up into the brain and other tissues for further metabolism, or, as discussed later, oxidized—principally in liver and kidney—to form betaine, a source of methyl groups. This latter process involves two enzymes, choline dehydrogenase and betaine dehydrogenase, usually collectively termed "choline oxidase" (❯ *Figure 3.2-2*). Negligible choline dehydrogenase activity (<1% of that found in liver and kidney) was observed in rat brain in vitro (Haubrich et al., 1979) and no evidence exists that the enzyme in brain actually converts choline to betaine in vivo; hence, we do not include the choline oxidase system among the brain proteins, discussed later, that interact directly with choline. Humans (Buchman

Figure 3.2-5

Phospholipases that metabolize phosphatidylcholine (PC). (a) Phospholipase D; (b) Phospholipase A2; (c) Phospholipase C. Boxes surrounding the portions of the PC molecule differentiate the glycerol, fatty acid (R1 and R2), and choline moieties. Lyso-PC, Lyso-Phosphatidylcholine; LPCAT, Lyso-Phosphatidylcholine acyltransferase; GPC-PD, Glycerophosphocholine phosphodiesterase; GPC-CPD, Glycerophosphocholine cholinephosphodiesterase; DAG, Diacylglycerol



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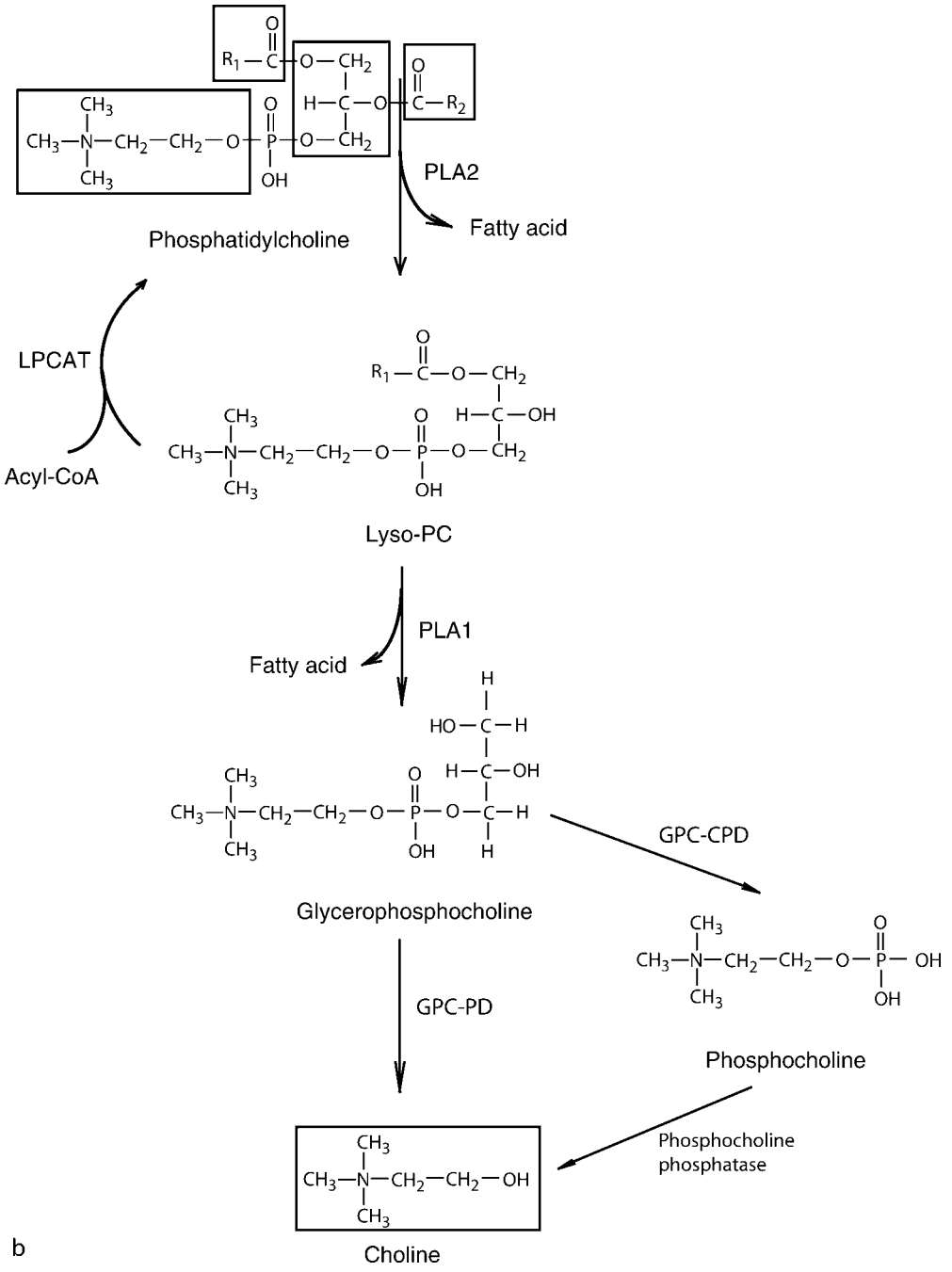

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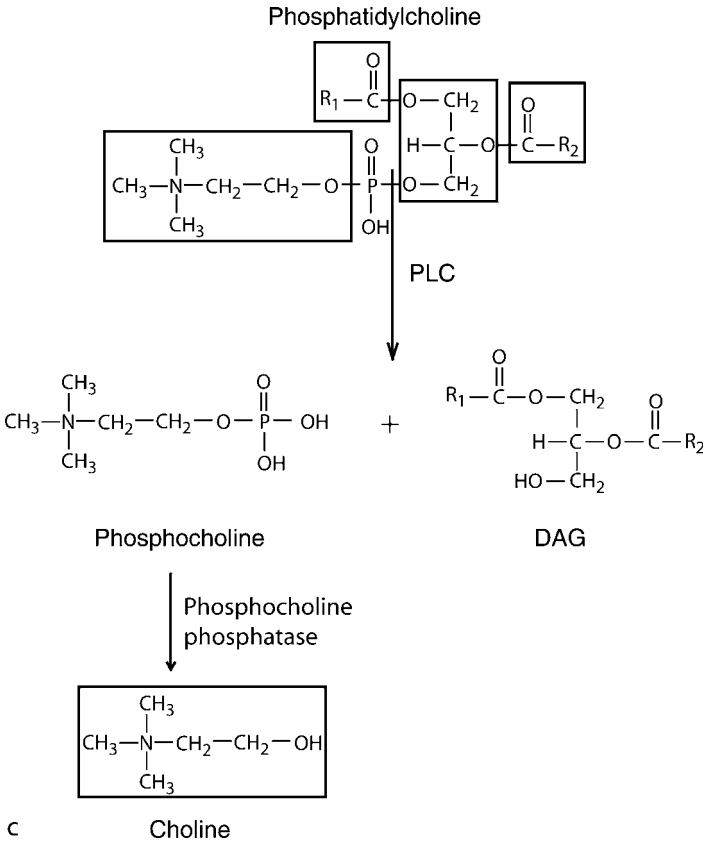


et al., 1994) and laboratory animals (Acara and Rennick, 1973; Acara et al., 1979) also excrete small amounts of unchanged choline into the urine (*Table 3.2-1*) by glomerular filtration followed by partial renal tubular reabsorbtion. Dietary choline or choline secreted into the gut can be broken down by intestinal bacteria to form trimethylamine and related amine products (de la Huerga and Popper, 1951; Neill et al., 1978). This process is responsible for the "fishy odor" sometimes detected in people taking large doses of choline supplements (Rehman, 1999).

2.2.1 Choline as a Source of Methyl Groups (Choline Oxidase System)

The choline oxidase system in mammals is composed of two enzymes; in microorganisms a single enzyme, also termed "choline oxidase" (EC 1.1.3.17) converts choline to betaine. In mammals, the choline is first oxidized to betaine aldehyde by choline dehydrogenase (EC 1.1.99.1) (*Figure 3.2-2*), an enzyme located at (or bound to) the inner membrane of mitochondria (Streumer-Svobodova and Drahota, 1977; Lin and Wu, 1986). This enzyme can also convert the aldehyde to betaine; however, unlike the choline oxidase of microorganisms, its affinity for betaine aldehyde is very low (only about 5% its affinity for choline), so choline dehydrogenase has only a minor effect on net betaine synthesis (Tsuge et al., 1980). This enzyme is a monomeric flavoprotein with a molecular weight of 61.000 Da (Lin and Wu, 1986); its activity requires FAD (Rothschild et al., 1954) and molecular oxygen serves as the primary electron acceptor (Zhang et al., 1992).

Table 3.2-4

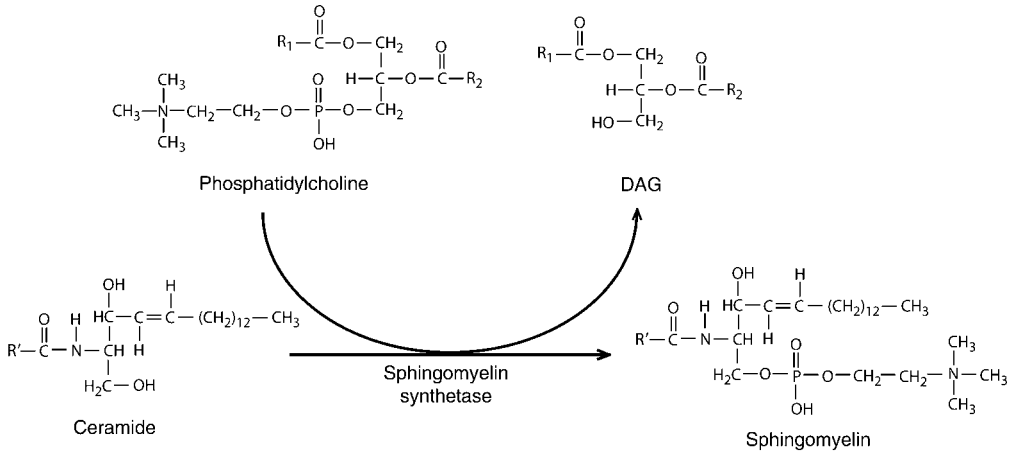
Effects of physiologic or pathologic conditions on circulating free choline concentrations in humans

Physiopathologic state	Free choline (μM)	Sample	References
Newborns			
Preterm newborns (0–2 days)	21.2–33.3	S	Buchman et al. (2001); Ilcol et al. (2005a)
Newborns at term (0–2 days)	28.1–68.1	S	Zeisel et al. (1980a); Buchman et al. (2001); Ilcol et al. (2005a)
Infants			
At postnatal age 3–28 days	24.2–31.5	S	Ilcol et al. (2005a)
At postnatal age 31–365 days	16.3–20.9	S	Ilcol et al. (2005a)
At postnatal age 366–730 days	12.8–16.3	P + S	Buchman et al. (2001); Ilcol et al. (2005a)
Children (2–12 years old)	10.9–12.9	S	Ilcol et al., 2005a
Adults (20–65 years old)	9.5–13.3	P + S	Savendahl et al. (1997); Ilcol et al. (2002b, 2005a)
1-week starved adults	7.5 ± 0.3	P + S	Savendahl et al. (1997)
Nonpregnant women	10.6 ± 0.5	S	Ilcol et al. (2002e)
Pregnant women	14.5–17.1	S	Ulus et al. (1998); Ilcol et al. (2002e)
Nonlactating women	10.6 ± 0.6	S	Ilcol et al. (2005a)
Breast-feeding lactating-women	14.7–19.2	S	Ilcol et al. (2005a)
Childbirth			
Before childbirth	15.4–16.1	P + S	Ulus et al. (1998); Ilcol et al. (2002e)
After (6 h) childbirth	9.9–10.8	P + S	Ulus et al. (1998); Ilcol et al. (2002e)
Marathon runners			
Before race	9.6–19.2	P	Conlay et al. (1986, 1992); Buchman et al. (1999, 2000b)
After (1–24 h) race	6.2–14.1	P	Conlay et al. (1986, 1992); Buchman et al. (1999, 2000b)
End-stage renal disease patients	32.5–44.2	P + S	Buchman et al. (2000b); Ilcol et al. (2002a, b)
Surgical patients			
Before surgery	10.7–12.3	P + S	Ulus et al. (1998); Ilcol et al. (2002d, 2004, 2006)
After (1–24 h) surgery	5.6–8.8	P + S	Ulus et al. (1998); Ilcol et al. (2002d, 2004, 2006)
Alzheimer's disease patients	10.4–11.1	P	Greenwald et al. (1985); Kanof et al. (1985)
Bipolar-manic patients	12.1–12.3	P	Jope et al. (1980); Stoll et al. (1991)
Alzheimer's disease patients	40.7–48.1	RBC	Greenwald et al. (1985); Kanof et al. (1985)
Bipolar-manic patients	23.9–106	RBC	Jope et al. (1980, 1984, 1985a, 1986); Stoll et al. (1991)
Unipolar-depressed patients	17–51	RBC	Wood et al. (1983); Jope et al. (1985b); Stoll et al. (1991)
Schizophrenic patients	16.7–90	RBC	Kuchel et al. (1984); Jope et al. (1985b); Stoll et al. (1991)
Patients under lithium treatment	277–626	RBC	Jope et al. (1980, 1984, 1985a, 1986); Stoll et al. (1991)
Healthy adults	15.8–22.6	Blood	Hasegawa et al. (1982); Danne et al. (2003)
Patients with noncardiac chest pain	19.9 ± 0.7	Blood	Danne et al. (2003)
Patients with unstable angina	24.5–47.4	Blood	Danne et al. (2003)

Note: Values are given as the range of the means from the cited references, or as the mean ± SEM

P, plasma; S, serum; RBC, red blood cell; Blood, whole blood

Figure 3.2-6
Biosynthesis of sphingomyelin (SM) from phosphatidylcholine (PC) and ceramide



One molecule of choline oxidized through the respiratory chain yields two molecules of mitochondrial ATP (Lin and Wu, 1986). Choline dehydrogenase has been cloned from rat liver mitochondria using a cDNA formed from the enzyme's terminal amino acid sequence (Huang and Lin, 2003). The enzyme is most active in liver and kidney (Bernheim and Bernheim, 1933; Mann and Quastel, 1937), and, as discussed earlier, only negligible choline dehydrogenase activity is observed in brain (Haubrich et al., 1979; Haubrich and Gerber, 1981). Recent estimates of choline dehydrogenase's K_m for choline—140–270 μM (Zhang et al., 1992)—suggest values that are substantially lower than those estimated earlier (5–7 mM; Rendina and Singer, 1959; Tsuge et al., 1980; Haubrich and Gerber, 1981). However, this revised K_m is still high compared with actual liver choline concentrations (60–230 μM; Sundler et al., 1972; Haubrich and Gerber, 1981), suggesting that treatments that increase hepatic choline levels also increase its rate of degradation. Very high portal venous choline concentrations (~2.5 mM; Zeisel et al., 1980b) produced experimentally in studies on isolated perfused livers can fully saturate the enzyme.

Betaine aldehyde dehydrogenase (EC 1.2.1.8) further oxidizes betaine aldehyde to betaine (*Figure 3.2-2*). This enzyme, found both in cytoplasm and mitochondria (Wilken et al., 1970; Pietruszko and Chern, 2001), uses NAD as a cofactor (Wilken et al., 1970). Its K_m for betaine aldehyde (118 μM in rat liver mitochondria, Chern and Pietruszko, 1995; 123 μM in rat liver cytoplasm, Vaz et al., 2000; 182 μM in human liver cytoplasm, Vaz et al., 2000) is probably substantially higher than actual in vivo concentrations of the aldehyde; hence, the betaine aldehyde formed when hepatic choline levels rise is rapidly metabolized.

Choline's oxidation occurs through two irreversible reactions; hence, betaine cannot be reduced back to choline. Choline administration enhances betaine formation both in vivo (Haubrich et al., 1975; Zeisel and Wurtman, 1981) and in vitro (Wilken et al., 1970; Zeisel et al., 1980b; Zhang et al., 1992). In isolated perfused liver, betaine accounts for ~60% of the labeled metabolites of [methyl-^{14}C]choline at concentrations of 5–125 μM (Zeisel et al., 1980b). Betaine is used by liver as a source of methyl groups for the generation of methionine from homocysteine, a process catalyzed by the enzyme betaine homocysteine methyltransferase (EC 2.1.1.5; Klee et al., 1961). The importance of this pathway (and thus of choline as a source of methyl groups) is probably increased when other methylating pathways have been compromised by ethanol ingestion, drugs, or nutritional deficiencies affecting folic acid, pyridoxine, or vitamin B12 (Barak and Tuma, 1983). Thus, treatment with betaine could be beneficial in early-stage alcoholic liver injury (Barak et al., 1996; Kharbanda et al., 2005).

2.3 Effects of Physiologic or Pathologic States on Plasma Choline

In adult humans, plasma (or serum) concentrations of free choline are maintained around 10 μM after a 6–24 h fast (*Table 3.2-4*), and increased by up to 50 μM postprandially, depending on the choline contents of the consumed food (Hirsch et al., 1978; Zeisel et al., 1980c). Basal plasma concentrations of free choline decrease by about 25–30% in human subjects undergoing a 1-week fast (Savendahl et al., 1997; da Costa et al., 2004) or consuming a choline-deficient diet (<50 mg/day) for 3–6 weeks (Zeisel et al., 1991; da Costa et al., 2006). Prolonged consumption of such diets can be associated with liver or muscle damage (da Costa et al., 2004, 2006).

A number of physiologic or physiopathological circumstances (e.g., infancy, pregnancy, lactation, surgery, parturition, marathon running, hemodialysis, and end-stage renal disease) can alter baseline plasma choline concentrations in humans (*Table 3.2-4*). These concentrations are much higher in newborns (about 40 μM) than in adult humans (Zeisel et al., 1980a; Zeisel and Wurtman, 1981; Buchman et al., 2001; Ilcol et al., 2002e, 2005a); a similar age relationship has been described in rats (Zeisel et al., 1980a; Zeisel and Wurtman, 1981) and rabbits (Zeisel et al., 1980a). In rats, the high postnatal choline concentrations fall to adult levels during the first 3 weeks of postnatal life (Zeisel and Wurtman, 1981); in humans, this occurs by 2 years of age (Ilcol et al., 2005a).

In humans, serum-free choline levels rise gradually during pregnancy (Ilcol et al., 2002e; Velzing-Aarts et al., 2005) reaching 15–20 μM at term (Ulus et al., 1998; Ilcol et al., 2002e, 2005a); then they decrease by 35–40% during the 12–20 h after delivery (Ulus et al., 1998; Ilcol et al., 2002e). Like pregnancy, breast-feeding is associated with elevated serum-free choline levels, in the mothers, rising to 15–20 μM during a 180-day period of lactation. In both pregnancy and lactation, considerable amounts of free choline are transferred from the mother to the fetus or breast-fed infant, through the placenta or the breastmilk, and used for growth and development (e.g., as a precursor of membrane phosphatides; Zeisel, 2006). The high-serum-free choline concentrations observed in pregnant and lactating women may reflect a process for promoting fetal and infant growth at the expense of depleting the mother's choline stores (Zeisel et al., 1995).

In patients with end-stage renal disease, plasma-free choline levels are several-folds higher than those in control subjects (Rennick et al., 1976; Buchman et al., 2000b; Ilcol et al., 2002a, b) or in patients who have successfully undergone renal transplantation (Ilcol et al., 2002a). Considerable amounts of choline are lost into hemodialysates (Rennick et al., 1976; Buchman et al., 2000b; Ilcol et al., 2002a) or peritoneal dialysates (Ilcol et al., 2002b), but plasma-free choline levels decrease only slightly during hemodialysis (Rennick et al., 1976; Buchman et al., 2000b; Ilcol et al., 2002a).

Several studies have shown that plasma-free choline concentrations decrease significantly by about 25–40% after prolonged exercise, e.g., running a marathon (Conlay et al., 1986, 1992; Buchman et al., 1999, 2000a), and remain depressed for at least 48 h after the race (Buchman et al., 1999).

Serum-free choline concentrations decrease by 20–45% during (Ulus et al., 1998; Ilcol et al., 2002d) and after surgery, in humans (Ulus et al., 1998; Ilcol et al., 2002d, 2004, 2006) or dogs (Ilcol et al., 2003b). This phenomenon is a response to surgical stress and is inversely correlated with the stress-induced elevations in serum cortisol, adrenocorticotropic hormone (ACTH), prolactin, and β-endorphin (Ilcol et al., 2002a). The magnitude and duration of surgery-induced declines in serum choline depend on the severity and the type of surgery (Ulus et al., 1998; Ilcol et al., 2002d, 2003b, 2005b). Thus, free choline concentrations return to presurgical values within 24 or 48 h after a cesarean section (Ilcol et al., 2002e), or a transurethral prostatectomy (Ulus et al., 1998), but require 72 h to do so after abdominal surgery (Ilcol et al., 2003b) or 96 h after coronary artery bypass surgery (Ilcol et al., 2004, 2006) or removal of a brain tumor (Ilcol et al., 2004). The decline in serum-free choline concentration associated with surgery can be mimicked in dogs by the administration of methylprednisolone (Ilcol et al., 2003b).

Perhaps paradoxically, plasma and whole blood choline concentrations reportedly increase significantly in patients with acute coronary syndromes (Danne et al., 2003, 2005).

3 Choline in the Brain

Because choline is, by virtue of its quaternary nitrogen atom, relatively polar, it had generally been assumed (Ansell and Spanner, 1971; Diamond, 1971) that plasma choline was unavailable to the brain. Moreover, as brain cells were also thought to be incapable of synthesizing choline de novo, the ability of cholinergic neurons to maintain the intracellular choline concentrations needed for ACh synthesis was usually attributed either to an extraordinarily effective reuptake mechanism, described later, for reutilizing virtually all the choline formed from the hydrolysis of ACh, or, less likely, to the uptake into brain of circulating PC or lyso-PC (Illingworth and Portman, 1972; Kuhar and Murrin, 1978). In addition, since the poor affinity of ChAT, the enzyme that catalyzes choline's conversion to ACh for choline made it likely that intracellular choline concentrations would control brain ACh synthesis; it was broadly conjectured that choline's high-affinity uptake from the synaptic cleft controlled ACh synthesis (cf., Taylor and Brown, 2006).

It is no longer held that the brain choline levels are sustained solely by the high-affinity uptake of free choline from synapses, or that variations in this uptake are normally responsible for observed variations in brain choline levels. Choline molecules (but not those of PC or lyso-PC; Pardridge et al., 1979) do readily cross the BBB (Cornford et al., 1978), and brain cells do indeed synthesize choline de novo (Blusztajn and Wurtman, 1981). Physiological variations do occur in choline levels within brain neurons; however, these result principally from changes in plasma choline concentrations after eating choline-rich foods, or from choline's metabolism. It is possible in laboratory studies to make the reuptake of intrasynaptic choline become the limiting factor controlling ACh synthesis, for example, by giving a drug-like hemicholinium-3 (HC3), which blocks the reuptake process. However, no food constituents or endogenous compounds have ever been shown to share this ability. It is possible that the density of choline-reuptake sites in nerve terminals may be modulated by the rate of ACh release (Taylor and Brown, 2006); however, variations in the rate of ACh release have not been demonstrated to affect the efficiency of choline reuptake.

Mammalian brains contain choline as the free base; as such water-soluble phosphorylated metabolites as phosphocholine and GPC (Nitsch et al., 1992), and as constituents of membrane phospholipids including PC, SM, and lyso-PC. Free choline levels in the brains of humans and rats reportedly vary between 36–44 μM (Ross et al., 1997) and 30–60 μM (Stavinoha and Weintraub, 1974; Klein et al., 1993), whereas PC and SM levels are orders of magnitude higher (∼2000–2500 μM and 250 μM, respectively; Marshall et al., 1996). These high levels reflect the ubiquity of phospholipids, and the numerous essential roles they mediate when they form membranes. Membrane phospholipids also serve as reservoirs for choline and for such "second messenger" molecules as DAG, AA, inositol trisphosphate (IP3), and phosphatidic acid.

3.1 Sources of Brain Choline

Free choline molecules in the brain derive from four known sources such as uptake from the plasma; liberation from the PC in brain membranes; high-affinity uptake from the synaptic cleft after ACh released from a cholinergic terminal has been hydrolyzed; and, probably to a minor extent, the breakdown of newly synthesized PC formed from the methylation of PE.

3.1.1 Uptake of Circulating Choline into the Brain

The brain can obtain circulating choline and various other circulating nutrients (e.g., neutral and basic amino acids, glucose, adenine, or adenosine; Pardridge and Oldendorf, 1977; Pardridge, 1986) via two routes: Small amounts can pass from the blood to the cerebrospinal fluid through the action of a specific transport protein, organic cation transporter 2 (OCT2), which is present in cells that line the choroid plexus (CP) (Sweet et al., 2001). However, orders of magnitude more pass bidirectionally between the blood and

the brain's extracellular fluid (ECF) by facilitated diffusion. This process is catalyzed by a different transport protein, not yet cloned, which is localized within the endothelial cells that line the brain's capillaries (Oldendorf and Braun, 1976; Pardridge and Oldendorf, 1977; Cornford et al., 1978). Its action is independent of sodium, and can be blocked by HC3.

Studies using an in situ brain perfusion technique, or a cell line of immortalized endothelial microvessels from rat brain (RBE4), have demonstrated the existence of a transport protein with a relatively low K_m for choline [(39–42 μM; Allen and Smith, 2001) or (20 μM; Friedrich et al., 2001)] which could mediate choline's bidirectional flux across the BBB. Other investigators using other experimental systems had proposed substantially higher K_ms for endothelial choline transport, i.e., 220–450 μM (Oldendorf and Braun, 1976; Pardridge and Oldendorf, 1977; Cornford et al., 1978; Mooradian, 1988). The differences among the affinities noted in these studies might, as discussed later, reflect the different methods used for their measurement. However, in any case, the protein would still be unsaturated at physiological plasma choline concentrations, and its net activity is still affected by variations in these concentrations. It might constitute a kind of pore through which choline can pass in either direction, based on the gradient between its blood and brain levels (Klein et al., 1990). Hence when plasma choline levels have been elevated, for example by eating a choline-rich meal (e.g., to 50 μM in the rat; Zeisel et al., 1980a), choline tends to enter the brain, but when plasma choline levels are low its flux is in the opposite direction. It has been estimated that the plasma choline concentration in rats required in order for the net choline flux to be from blood to brain is about 15 μM; below this concentration, net choline flux presumably is from brain to blood (Klein et al., 1990).

No endogenous circulating compound has been shown to compete effectively with choline for facilitated diffusion across the BBB. Very high concentrations of carnitine and spermidine, compared with those in the blood, can reduce brain uptake of choline by 20–25% (Cornford et al., 1978). One drug, diethylaminoethanol, apparently does block BBB choline uptake, and has been used to lower brain choline levels and thereby suppress ACh synthesis (Cornford et al., 1978; Millington et al., 1978). Lithium ion, given acutely (Cornford et al., 1978) or chronically (Millington et al., 1978), may also block BBB choline uptake. However, lithium also blocks choline's efflux from brain to blood, thus producing a net increase in brain choline levels (Millington et al., 1978).

Once circulating choline has entered the brain's ECF, it can be taken up into all cells by a low-affinity transport protein (K_m=30–100 μM), or into cholinergic nerve terminals by a high-affinity uptake protein (K_m=0.1–10 μM) (Haga and Noda, 1973; Yamamura and Snyder, 1973; Blusztajn and Wurtman, 1983). Both of these are described later. The high-affinity process, unlike the passage of choline across the BBB, is energy- and sodium-dependent.

3.1.2 Liberation from Membrane PC

The choline in membrane PC can be liberated through the actions of the phospholipase enzymes, described earlier, which catalyze the hydrolysis of various bonds between PC's three oxygen molecules and fatty acids or its phosphate moiety (❯ *Figure 3.2-5*). The activation of each of these enzymes is tightly regulated and, in general, initiated by the interaction of a neurotransmitter or other biologic signal with a receptor coupled to a G-protein. For example, both the PLC enzymes (which act on PC to yield DAG and phosphocholine, or on PI) and PLD (which acts on PC to yield phosphatidic acid and choline) are activated when ACh attaches to M1 or M3 muscarinic receptors (Sandmann and Wurtman, 1990, 1991; Sandmann et al., 1991). The DAG generated by PLC activates a family of protein kinase (PK) enzymes that phosphorylate various proteins, including those that control the metabolism of the amyloid precursor protein (APP) to form either soluble APP or the A-beta peptides (Hung et al., 1993; Nitsch et al., 1994; Slack et al., 1997; Slack and Wurtman, 2006).

The release of choline from PC can also be enhanced, and its reincorporation into PC is diminished by sustained neuronal depolarization (Farber et al., 1996). This process has been termed "autocannibalism" when some of the choline is diverted for the synthesis of ACh (Blusztajn et al., 1986; Ulus et al., 1989).

Autocannibalism may, by decreasing the quantities of phosphatide molecules, and thus of neuronal membranes, underlie the particular vulnerability of cholinergic neurons in certain diseases (Blusztajn et al., 1986; Ulus et al., 1989). It is not known whether the accelerated breakdown of PC associated with sustained neuronal depolarization results from changes in ion flux or requires the release of local neurotransmitters and activation of particular receptors. The depletion of membrane PC and other phosphatides—including those not containing choline—caused by frequent or sustained depolarizations can be diminished or blocked entirely, and the release of ACh is enhanced by providing the brain with supplemental choline (Ulus et al., 1989).

3.1.3 Reutilization of Choline Formed from Hydrolysis of Acetylcholine

ACh released into synapses is rapidly hydrolyzed to free choline and acetate. This process terminates the neurotransmitter's physiologic actions, i.e., its ability to combine with and activate its pre- or postsynaptic muscarinic or nicotinic receptors. (The inactivation of ACh differs from that of other aminergic transmitters, e.g., dopamine and serotonin, in which it involves a chemical change in the neurotransmitter molecule, and not simply physical removal of that molecule from the synaptic cleft by reuptake into its nerve terminal of origin.) The enzymes that catalyze ACh hydrolysis, the acetylcholinesterases (EC 3.1.1.7; AChE), are particularly abundant within the cholinergic synapse; they are synthesized in the cholinergic neuron and secreted into the synapse, along with ACh, when the neuron is depolarized. A related enzyme, butyrylcholinesterase (EC 3.1.1.8; BuChE), synthesized in the liver and present in plasma, probably functions to inactivate potentially toxic dietary esters but not intrasynaptic ACh: It is active in the nervous system during development, but not thereafter, and mutant animals lacking the BuChE gene—in contrast to those lacking AChE—apparently fail to exhibit neurologic symptoms (Taylor and Radic, 1994; Giacobini, 2003).

Most of the free choline liberated by the intrasynaptic hydrolysis of ACh is taken back up into its nerve terminal of origin by the high-affinity choline transporter (CHT) described later, and either reacetylated to form ACh or phosphorylated for ultimate conversion to membrane PC (Ulus et al., 1989).

3.1.4 De Novo Synthesis of Phosphatidylcholine and Choline

As described earlier, brain cells—including nerve terminals (Holbrook and Wurtman, 1988)—contain all the enzymes needed to synthesize PC from ethanolamine (❯ *Figure 3.2-3*) or from PS. These include the Kennedy cycle enzymes that convert ethanolamine to PE (Spanner and Ansell, 1979), PS decarboxylase, which forms PE from PS (Butler and Morell, 1983), and the enzymes (PEMT1 and PEMT2), which methylate PE (Crews et al., 1980).

4 Brain Proteins that Interact with Choline

Free choline is known to interact with two brain enzymes and four transport proteins, as well as various receptors for ACh.

The two enzymes are ChAT and CK—which catalyze, respectively, the transformations of choline to ACh within cholinergic terminals, and to phosphocholine within all cells.

The four transport proteins include two that move choline across the BBB, i.e., the facilitated-diffusion site in brain capillaries through which choline passes, bidirectionally, between the plasma and the brain's ECF, and the organic cation transporter that carries plasma choline across the CP and into the cerebrospinal fluid; and two that enable the choline in brain ECF to enter cells, i.e., the low-affinity uptake site that catalyzes choline's uptake into all brain cells, and the high-affinity uptake site that transports intrasynaptic choline into the presynaptic terminals of cholinergic neurons.

The cholinergic receptors, which choline can also activate, include both nicotinic and muscarinic varieties. This section describes the properties of each of these proteins, and the consequences of their interactions with choline.

4.1 Enzymes

4.1.1 Choline Acetyltransferase

ChAT (acetyl-CoA: choline-O-acetyltransferase, EC 2.3.1.6) mediates a single reaction, the transfer of an acetyl group from acetyl-coenzyme A (acetyl-coA) to choline, which thereby generates the neurotransmitter ACh in cholinergic neurons.

ChAT, a single-stranded globular protein, is encoded by a single gene with, in humans, six distinct transcripts formed from the alternative splicing of five noncoding exons (Misawa et al., 1992, 1997; Oda et al., 1992; Robert and Quirin-Stricker, 2001) Polymorphism among these transcripts is apparently limited to their 5′-untranslated regions. In humans, four of the six transcripts (designated as H, R, N1, and N2) translate to the same 69-kD protein (Misawa et al., 1992, 1997; Oda et al., 1992; Robert and Quirin-Stricker, 2001). The fifth and sixth transcripts, designated as M and S, have two translation sites and yield, besides the 69-kD enzyme, 82-kD and 74-kD forms of ChAT, respectively. The 82-kD ChAT differs from the 69-kD form in that it has an aminoterminal extension with 118 amino acid residue (Oda et al., 1992; Misawa et al., 1997). Physiological roles for the 74-kD and 82-kD forms of ChAT remain to be elucidated, and indeed it is not clear that these larger forms of human ChAT actually are synthesized in vivo (Oda, 1999).

ChAT probably exists in at least two forms within cholinergic nerve terminals—a soluble form (80–90% of the total enzyme activity) and a membrane-associated form (10–20%; Benishin and Carroll, 1981; Salem et al., 1994; Pahud et al., 1998). These two forms exhibit different physicochemical and biochemical properties (Benishin and Carroll, 1983; Eder-Colli et al., 1986; Pahud et al., 2003). The soluble form is hydrophilic, and the membrane-bound form is amphiphilic (Benishin and Carroll, 1983; Eder-Colli et al., 1986; Pahud et al., 2003). Soluble ChAT has higher affinities for both of its substrates, choline and acetyl-CoA, when assayed at low ionic strength (K_m for choline 350 μM; for acetyl-CoA 2.5 μM) than that when assayed at higher ionic strengths (K_m for choline 6700 μM; for acetyl-CoA 77 μM; Rossier, 1977). The activity of ChAT in crude synaptosomal preparations (presumably representing a mixture of the soluble and membrane-bound forms) also varies with ionic strength; the affinity of synaptosomal ChAT for choline appears to be greater than that of soluble ChAT ($K_m = 22$ μM at low ionic strength and 540 μM at high ionic strength; Rossier, 1977). In any case, ChAT is invariably unsaturated with choline at the choline concentrations that could exist within nerve terminals (Tucek, 1990), indicating that ChAT is in kinetic excess (Hersh, 1982; Tucek, 1990), and that its substrate-saturation, not its levels, is rate limiting in ACh synthesis. Both choline and acetyl-CoA (Rossier, 1977; Hersh, 1982; Tucek, 1990) levels can affect the rate at which ACh is produced.

There is also evidence that phosphorylation and dephosphorylation of ChAT can alter its catalytic activity, subcellular distribution, and interactions with other cellular proteins (see review of Dobransky and Rylett, 2005). ChAT is a substrate for multiple PKs; 69 kDa ChAT is phosphorylated by PK-C, PK-CK2, and a Ca^{2+}/calmodulin-dependent PK-II (CaM-kinase) but not by PK-A, whereas 82 kDa ChAT is phosphorylated by PK-C and CaM-kinase (Dobransky et al., 2000, 2001). ChAT is differentially phosphorylated by PK-C isoforms on four of its serine residues (Ser-440, Ser-346, Ser-347, and Ser-476) and one threonine residue (Thr-255); this phosphorylation is hierarchical, such that phosphorylation at Ser-476 is required in order for the other serines to become phosphorylated (Dobransky et al., 2004). Phosphorylation at some but not all of those sites (Ser-476 with Ser-440 and Ser-346/347; Dobransky et al., 2004) affects basal ChAT activity. Phosphorylation of ChAT by PK-C alone can double the enzyme's activity, whereas coordinated phosphorylation of ChAT at threonine 456 (by CaM-kinase II) and serine 440 (by PK-C) can treble ChAT activity (Dobransky et al., 2003). Whether the phosphorylation and dephosphorylation of ChAT also alter the enzyme's affinities for choline or acetyl-CoA in intact cells is clear.

4.1.2 Choline Kinase

CK (ATP:choline phosphotransferase; EC 2.7.1.32) catalyzes the first phosphorylation reaction in the Kennedy cycle of PC synthesis (*Figure 3.2-4*); ATP is the phosphate donor and the presence of Mg^{+2} is required (Wittenberg and Kornberg, 1953). CK can also catalyze the phosphorylation of ethanolamine, as well as N-monomethylethanolamine and N,N-dimethylethanolamine (Ishidate et al., 1985; Porter and Kent, 1990; Uchida and Yamashita, 1990); however, a separate ethanolamine kinase enzyme exists, demonstrated by cloning cDNA from human liver (Lykidis et al., 2001). CK is mainly cytosolic but is also associated with particulate (membrane-bound) fractions of rat striatum (Reinhardt and Wecker, 1983). The enzyme has been purified to homogeneity from various rat tissues (Ishidate et al., 1985; Porter and Kent, 1990), including brain (Uchida and Yamashita, 1990). HC3 (Ansell and Spanner, 1974) and ADP (Burt and Brody, 1975) inhibit CK activity in vitro, whereas high concentrations of the polyamines spermine and spermidine (Uchida and Yamashita, 1990) enhance its activity. Several isoforms of CK exist in brain and other tissues, differentiable by their subunit masses (Porter and Kent, 1990; Uchida and Yamashita, 1990) and by cloning and expression studies (Uchida and Yamashita, 1992; Uchida, 1994; Aoyama et al., 1998). At least as three isoforms (CK-α1, CK-α2, and CK-β), encoded by two separate genes termed *ck-α* and *ck-β* (Aoyama et al., 1998, 2000, 2004), are now recognized. The latter resides on chromosome 22q13 in humans (Froguel and McGarry, 1997); the locus of the former awaits determination. These isoforms may not be active as monomers, but become active on forming dimeric or oligomeric structures (Aoyama et al., 2004).

In some circumstances, CK activity may be rate limiting in PC synthesis; for example, a 3.5-fold increase in CK activity in livers of rats deficient in essential fatty acids was accompanied by a parallel increase in PC synthesis (Infante and Kinsella, 1978). Similar relationships have been described in livers of estrogen-treated roosters (Vigo and Vance, 1981) or quiescent murine 3T3 cells in culture (Warden and Friedkin, 1985). However, it is probably not the activity of CK per se, but rather its degree of substrate saturation that affects the rate of PC synthesis. The K_m of CK for choline in rat brain is 14–134 μM (Uchida and Yamashita, 1990; Cao and Kanfer, 1995); this value is 32–310 μM in rabbit brain (Haubrich, 1973). An even higher K_m value (2.6 mM) was described by Spanner and Ansell (1979) who assayed the enzyme at a more physiological pH (7.5) than that customarily used (pH = 9.0); this allowed phosphocholine, CK's reaction product, to be assayed without first being hydrolyzed. Hence, CK is unsaturated with choline at normal brain choline concentrations (30–60 μM), and the production of phosphocholine through CK, like that of ACh by ChAT, is controlled principally by brain choline levels (Millington and Wurtman, 1982; Cohen et al., 1995).

4.2 Transport Proteins

4.2.1 Facilitated-Diffusion Carrier at Blood–Brain Barrier

A transport protein that mediates the bidirectional facilitated-diffusion of choline at the BBB has been identified (Oldendorf and Braun, 1976; Pardridge and Oldendorf, 1977; Cornford et al., 1978). This protein does not require metabolic energy or sodium flux and cannot maintain a concentration gradient.

The CHT at the BBB, as discussed earlier, might allow bidirectional passage of choline based on the gradient between its blood and brain concentrations (Klein et al., 1990). A blood choline concentration of 15 μM has been estimated to be required for choline influx to the rat's brain to predominate; below this concentration, choline efflux predominates (Klein et al., 1990).

As discussed earlier, the BBB CHT's K_m for choline in vivo is estimated as 220–450 μM (Oldendorf and Braun, 1976; Pardridge and Oldendorf, 1977; Cornford et al., 1978; Mooradian, 1988). Hence, it is unsaturated at physiologic plasma choline concentrations (~10 μM). The K_m of BBB CHT for choline has been demonstrated as 39–42 μM in a recent study using an in situ brain perfusion technique (Allen and Smith, 2001); the higher affinity of the BBB transporter for choline found in this study, compared with previous data, probably reflects methodological differences. In vitro assays, using cell lines of immortalized rat (RBE4; Friedrich et al., 2001) and mouse (MBE4; Sawada et al., 1999) brain endothelial microvessels, have demonstrated the existence of a transport protein with a relatively low K_m for choline (20 μM in both

studies). However, these studies investigated uptake only at the luminal side of the endothelial cells, and not at both luminal and abluminal sides as was investigated in previous in vivo studies. The CHT at the BBB has not yet been cloned and awaits further characterization. A perhaps-related transporter for carnitine, the organic cation/carnitine transporter OCTN2 (Tamai et al., 1998), has been cloned from RBE4 cells (Friedrich et al., 2003). Carnitine uptake through this transporter is not blocked by choline (Tamai et al., 1998).

4.2.2 Choroid Plexus Choline Transporter

Much less blood choline is transported through the CP epithelium to the cerebrospinal fluid than through the BBB carrier to the brain's ECF, because the surface area of the CP epithelium is much smaller than that of the BBB epithelium (Pardridge, 2001). Ventricular choline transport, in the rat, is mediated by one of the OCT proteins, OCT2 (Sweet et al., 2001). The three subtypes of OCTs (OCT1–3) have been isolated from rat (Grundemann et al., 1994; Okuda et al., 1996; Kekuda et al., 1998), mouse (Schweifer and Barlow, 1996; Mooslehner and Allen, 1999), and human tissues (Gorboulev et al., 1997; Zhang et al., 1997; Grundemann et al., 1998). OCTs are transmembrane proteins with 12 membrane-spanning domains (Koepsell et al., 2003). Transport of a cation by an OCT protein is electrogenic, Na^+-independent, and reversible with respect to direction (Koepsell and Endou, 2004). In the human, genes encoding OCT1–3 have been found on chromosome 6 (6q26–6q27; Koehler et al., 1997, 2003). The hOCT2 protein is expressed in kidney (Gorboulev et al., 1997) and brain (Busch et al., 1998); however, its localization in human brain ventricles, and its possible transport activity remain to be established.

4.2.3 High-Affinity Uptake Protein in Cholinergic Terminals

A saturable, sodium-and energy-dependent, HC3-sensitive, high-affinity CHT has been demonstrated in synaptosomes (Yamamura and Snyder, 1972, 1973; Guyenet et al., 1973; Haga and Noda, 1973). The K_m of this transporter for choline is 0.1–10 μM (Guyenet et al., 1973; Haga and Noda, 1973; Yamamura and Snyder, 1973; Blusztajn and Wurtman, 1983). Choline uptake through the CHT is competitively inhibited by nanomolar concentrations of HC3 (K_i: 10–100 nM; Yamamura and Snyder, 1972; Haga and Noda, 1973; Kuhar and Murrin, 1978).

The high-affinity CHT protein, made up of two polypeptides with molecular masses of 58 and 35 kDa, has been identified and partially purified from rat corpus striatum (Rylett et al., 1996). cDNAs from rat (rCHT1; Okuda et al., 2000), mouse (mCHT1; Apparsundaram et al., 2001), and human (hCHT1; Apparsundaram et al., 2000) have also been isolated, cloned and expressed. These studies have shown that CHT1 does not belong to the neurotransmitter transporter family, but rather to the sodium-dependent glucose transporter family (SLC5, in which CHT1 is designated as SLC5A7) (Apparsundaram et al., 2000; Okuda et al., 2000; Okuda and Haga, 2003). CHT1 protein has 13 transmembrane domains (Apparsundaram et al., 2000; Okuda et al., 2000). The human CHT1 gene is localized on chromosome 2q12 (Apparsundaram et al., 2000).

High-affinity choline transport occurs predominantly into terminals of cholinergic neurons (Misawa et al., 2001). Using antibodies raised against CHT1, CHT-immunoreactive cells have been shown to be widely distributed throughout the rat, primate, and human central nervous systems (Misawa et al., 2001; Kus et al., 2003). Primate cerebellums contain numerous CHT-immunoreactive cells (Kus et al., 2003), and mouse cerebellum expresses CHT1 mRNA, particularly during development (Berse et al., 2005). CHT1 is also present in terminals and in those of peripheral motor neurons (Lips et al., 2002; Nakata et al., 2004) and of parasympathetic neurons to the tongue (Lips et al., 2002). CHT1 is not expressed in glial cells (Inazu et al., 2005) but, contrary to what had been believed, is expressed in such nonneuronal cells as rat trachea (Pfeil et al., 2003), rat and human arteries (Lips et al., 2003), and skin (Haberberger et al., 2002). The function of CHT1 in these tissues awaits determination.

Within neurons, CHT immunoreactivity is detectable in cell soma, proximal dendrites, axons and, particularly, axon terminals (Kus et al., 2003). Within the terminals, the CHT1 protein is especially

abundant in plasma membrane, synaptic vesicles, and endosomal vesicles (Ferguson et al., 2003; Ribeiro et al., 2003; Ferguson and Blakely, 2004). In motor neurons of diaphragm, the CHT is mainly (>90%) concentrated within synaptic vesicles, rather than in the presynaptic membrane itself (Nakata et al., 2004). These vesicles may store CHT1 in the resting state, and the protein may migrate to the synaptic membrane during depolarization (Ribeiro et al., 2003, 2005).

Activity-dependent modulation of CHT1 has been described in studies using several different experimental systems, for example electrical or pharmacological stimulation of cholinergic neurons in vitro (reviewed in Ferguson and Blakely, 2004). The capacity and density of CHTs are apparently increased in medial prefrontal cortices of rats performing attentional tasks (Apparsundaram et al., 2005). Neuronal activity per se (Ferguson et al., 2003) might, by altering the phosphorylation state of CHT1 protein (Gates et al., 2004), enhance the transfer of CHT1 into and out of vesicles, thus modulating its activity. NGF, which can upregulate CHT1 through a PI3K-dependent process, might similarly influence its activity (Berse et al., 2005).

4.2.4 Low-Affinity Cellular Uptake Protein

A nonsaturable, Na^+-independent, high-capacity, and low-affinity CHT has also been identified (Haga and Noda, 1973; Yamamura and Snyder, 1973). Not surprisingly—since all cells need choline for phospholipid synthesis—it appears to be ubiquitous in mammals and is found, in, for example, kidney (Bevan and Kinne, 1990), liver (Zeisel et al., 1980), and placenta (Grassl, 1994) as well as in brain synaptosomes (Ferguson et al., 1991). The K_m of the low-affinity transport protein for choline varies between 30 and 100 μM (Dowdall and Simon, 1973; Haga and Noda, 1973; Yamamura and Snyder, 1973); it also is inhibited by HC3 with K_is of about 40–50 μM (Haga and Noda, 1973) in brain and 100 μM in human placenta (Grassl, 1994). Low-affinity choline transport has been suggested to be a carrier-mediated process (Ferguson et al., 1991; Inazu et al., 2005). As discussed later, CTL1, a member of the family of choline-transporter-like proteins (Traiffort et al., 2005), has been proposed as mediating the low-affinity transport of choline into rat cortical astrocytes (Inazu et al., 2005) and mouse cortical neurons (Fujita et al., 2006).

The choline-transporter-like proteins CTL1–CTL5 are encoded by five different genes, also labeled from CTL1 to CTL5 (Traiffort et al., 2005). CTL1, a transmembrane protein with 10 transmembrane domains, has been cloned and characterized from rat (rCTL1; O'Regan et al., 2000), human (hCTL1; Wille et al., 2001), and mouse (mCTL1; Yuan et al., 2004) tissues. The human gene is located on chromosome 9q31.2 (Wille et al., 2001), and its protein product is expressed as two polypeptides, of 50 and 23 kDa, which have been found in such tissues as brain, heart, small intestine, kidney, liver, lung, skeletal muscle, pancreas, spleen, ovary, and testis (Yuan et al., 2006).

Another choline-transporting system, the OCT proteins (members of the solute carrier family SLC22; Koepsell et al., 2003; Koepsell and Endou, 2004), have also been implicated in low-affinity choline uptake. For example, rat OCT1 (rOCT1), cloned from renal proximal tubule epithelial cells or hepatocytes and expressed in Xenopus oocytes, can mediate low-affinity choline uptake (K_m = 1.1 mM; Busch et al., 1996), and human OCT1 (hOCT1) and human OCT2 (hOCT2) can mediate, respectively, hepatic and renal choline transport (K_m = 210 μM; Gorboulev et al., 1997).

It has not yet been determined whether the low-affinity CHT is one of the OCT proteins; CTL1 (perhaps more likely in brain; Inazu et al., 2005; Fujita et al., 2006); or even a different protein.

4.3 Receptors

Choline in sufficiently high concentrations can directly activate both muscarinic (mAChRs) and the nicotinic (nAChRs) acetylcholine receptors. The five muscarinic receptors (M1–M5) mediate slow metabolic responses to ACh, and the nicotinic receptors, which are ligand-gated ion channels, implement fast, ACh-mediated synaptic transmission in the CNS, ganglia, and neuromuscular synapses. The M1, M3, and

M5 muscarinic receptors activate phospholipase C, thereby generating the second messengers IP3 and DAG (Caulfield and Birdsall, 1998); the M2 and M4 muscarinic receptors inhibit adenylate cyclase activity, thus reducing intracellular cAMP levels, or can enhance the flux of potassium and other ions through nonselective ion channels. The nicotinic receptors, pentameric structures made up of combinations of 17 known individual subunits, increase the flux of sodium into postsynaptic cells, thus increasing the likelihood of the cells' depolarization. Free choline concentrations in synaptic fluid following neuronal depolarization apparently have not been measured, and may or may not attain levels sufficient to activate cholinergic receptors under physiological circumstances. Much higher concentrations, produced experimentally, are readily shown to activate the receptors in vitro.

Many years ago, it was noted that choline can produce "muscarine-like" or "nicotine-like" effects in various peripheral tissues (Dale, 1914; Chang and Gaddum, 1933), voluntary muscles (Bacq and Brown, 1937; Hutter, 1952; Del Castillo and Katz, 1957), and autonomic ganglia (Feldberg and Vartiainen, 1934; Kosterlitz et al., 1968; Krstic, 1972), with 1/20,000 to 1/714th the potency of ACh (Chang and Gaddum, 1933). Functional (Pomeroy and Raper, 1972; Ulus et al., 1979, 1988a; Holz and Senter, 1981) and receptor binding studies (Speth and Yamamura, 1979; Palacios and Kuhar, 1979; Costa and Murphy, 1984; Ulus et al., 1988), in which the choline presumably had not first been acetylated to authentic ACh, identified the effective choline concentrations needed to bind to AChRs and/or to produce ACh-like biological responses. Choline, acting as a direct muscarinic agonist, excited cortical neurons (Krnjevic and Reinhardt, 1979); contracted isolated smooth muscle in rat stomach fundus (EC50 = 0.41 mM), rat trachea (EC50 = 1.7 mM), rat urinary bladder (EC50 = 10.9 mM) (Ulus et al., 1988), and guinea pig ileum (EC = 0.6 mM; Pomeroy and Raper, 1972 or EC50 = 0.20 mM; Ulus et al., 1979); and reduced the frequency at which isolated rat or guinea pig right atrium beat spontaneously (Ulus et al., 1979, 1988). It also inhibited ACh release from myenteric plexus-longitudinal muscle preparations of guinea pig ileum (EC50 = 0.3 mM) (Kilbinger and Kruel, 1981), and inhibited [^{3}H]-quinuclidinyl benzilate binding to rat brain membranes (Placios and Kuhar, 1979; Speth and Yamamura, 1979; Costa and Murphy, 1984; Ulus et al., 1988) and rat peripheral tissues (Ulus et al., 1988). Choline's potency for inhibiting [^{3}H]-quinuclidinyl benzilate binding was found to vary among brain regions (K_i = 0.46–3.5 mM) (Palacios and Kuhar, 1979; Speth and Yamamura, 1979; Costa and Murphy, 1984; Ulus et al., 1988) and also in peripheral tissues (K_i = 0.28–1.17 mM) (Ulus et al., 1988). The wide range of variations in the muscarinic potency of choline and in its relative tissue selectivity (Ulus et al., 1988) may result from its varying affinities for mAChRs subtypes (M1–M5). Choline acts as a full agonist on human mutant M1 receptors to stimulate phosphoinositide hydrolysis (EC50 = 0.2 mM; Huang et al., 1998), and on cloned human M1 receptor to stimulate nitric oxide synthesis and elevate intracellular Ca^{+2}, at 0.1–1.0 mM concentrations (Carriere and El-Fakanay, 2000).

By activating nicotinic receptors as a full agonist (Ulus et al., 1988) and/or a partial agonist (Holz and Senter, 1981), choline stimulates catecholamine secretion from the vascularly perfused adrenal gland (EC50 = 2.1 mM; Ulus et al., 1988), and from primary cultures of bovine adrenal chromaffin cells (at 1–10 mM; Holz and Senter, 1981). It also competes with L-[^{3}H]-nicotine for binding to membrane preparations of rat brain (Costa and Murphy, 1984; Ulus et al., 1988) and peripheral tissues (Ulus et al., 1988). The potency of choline in displacing L-[^{3}H]-nicotine from brain nicotinic receptors varies within a threefold range (K_i = 379–1167 μM), and within a 1.5-fold range for peripheral tissues (K_i = 575–805 μM; Ulus et al., 1988). Patch-clamp studies have shown that choline interacts with nAChRs in a "subtype selective" and concentration-dependent manner. At concentrations of 0.1–10 mM, choline acts as a full agonist on α7 nAChR (Mandelzys et al., 1995; Papke et al., 1996, 2000; Alkondon et al., 1997, 2000; Albuquerque et al., 1998; Cuevas et al., 2000; Papke and Papke, 2002; Alkondon and Albuquerque, 2006; Gonzales-Rubio et al., 2006) or a partial agonist on α3β4 nAChRs (Mandelzys et al., 1995; Papke et al., 1996; Albuquerque et al., 1998), α3β4* nAChRs (Seddik et al., 2003), and α4β4 nAChRs (Zwart and Vijverberg, 2000). It desensitizes α7nAChRs at 10–100 μM concentrations (Mandelzys et al., 1995; Papke et al., 1996, 2000, 2002; Albuquerque et al., 1997; Alkondon et al., 1997), inhibits α3β4* and α4β2* nAChRs at 10–1000 μM concentrations (Alkondon and Albuquerque, 2006), and potentiates or inhibits α4β4 nAChR-mediated ACh currents at 10–300 μM or 1–30 mM concentrations, respectively (Zwart and Vijverberg, 2000).

5 Utilization of Choline in Brain

All cells use choline to produce the PC and SM in their membranes. Cholinergic neurons also use choline for an additional purpose, synthesis of their neurotransmitter, ACh. Both the PC and the ACh are ultimately broken down to regenerate free choline, thus both of these compounds can also be considered "reservoirs" for free choline. The synthesis of PC (*Figure 3.2-4*) is initiated by the phosphorylation of choline, catalyzed by an enzyme, CK, which forms phosphocholine by transferring a monophosphate group from ATP to the hydroxyl oxygen of the choline. As described later, this phosphocholine then combines with cytidine-5′-triphosphate (CTP) to form cytidine-5′-diphosphocholine (CDP-choline), which, in turn, combines with DAG to yield PC. The synthesis of ACh, catalyzed by the enzyme ChAT, involves a single reaction, the transfer of an acetyl group from acetyl-CoA, also to the hydroxyl oxygen of the choline. The ACh is then stored, largely within synaptic vesicles, for future release.

Both CK and ChAT have low affinities for their choline substrate: Their K_ms in brain, which describe the choline concentrations at which the enzymes operate at only half-maximal velocity, may be as high as 2.6 mM (Spanner and Ansell, 1979) and 540 μM (Rossier, 1977), respectively, whereas brain choline levels, as noted earlier, are only about 30–60 μM, and thus well below the concentrations that would probably be needed to enable either enzyme to operate at maximal velocity. Hence, both of the enzymes are highly responsive to treatments that raise or lower brain choline levels.

The ability of choline administration to increase the syntheses and brain levels of phosphocholine and ACh was first noted in 1982 (Millington and Wurtman, 1982) and 1975 (Cohen and Wurtman, 1975; Haubrich et al., 1975). It had previously been shown that the synthesis and levels of another brain neurotransmitter, serotonin, were increased if animals were given physiologic doses of its circulating precursor, tryptophan (Fernstrom and Wurtman, 1971; Cansev and Wurtman, 2006). This was because tryptophan hydroxylase, the enzyme that determines the overall rate at which tryptophan is converted to serotonin, has a low affinity for this substrate. Moreover, since ChAT's affinity for choline had also been shown, in vitro, to be low, it seemed reasonable to enquire into whether choline's ChAT-mediated conversion to ACh also was precursor-dependent. Once this relationship was affirmed, experiments soon followed demonstrating the precursor-dependence of phosphocholine synthesis (Millington and Wurtman, 1982).

Even though brain choline concentrations shared with those of tryptophan the ability to control the rates at which the precursor is of used for neurotransmitter synthesis, the two precursors differed in an important respect: Although tryptophan and choline are both used by certain neurons for two purposes: tryptophan for conversion to serotonin and incorporation into proteins, and choline for conversion to ACh and incorporation into phospholipids, in the case of tryptophan these two processes are segregated into different parts of the neuron—the nerve terminal and perikaryon, respectively—whereas for choline both can take place within the nerve terminal, inasmuch as that structure contains both ChAT and CK. Hence, the acetylation and phosphorylation of choline sometimes compete for available substrate (Farber et al., 1996; Ulus et al., 2006): When cholinergic neurons are forced to fire frequently and to sustain the release of ACh, choline's incorporation into PC decreases (Farber et al., 1996) and the breakdown of membrane PC increases ("autocannibalism"), liberating additional choline for ACh synthesis (Maire and Wurtman, 1985; Blusztajn et al., 1986; Ulus et al., 1989). When the utilization of choline to form PC is increased (by providing supplemental uridine and an omega-3 fatty acid; see later), ACh synthesis is not diminished, probably because so little choline is used for phosphatide formation compared with the amount used for ACh synthesis (Ulus et al., 2006).

5.1 Biosynthesis of Acetylcholine

ACh is synthesized in cholinergic neurons—principally their terminals—by the ChAT-mediated acetylation of free choline. Since, as described earlier, ChAT's affinity for choline is low compared with brain choline levels, local choline concentrations normally control the rate of ACh synthesis (Blusztajn and Wurtman, 1983), and treatments which increase brain choline (e.g., administering choline; Cohen and Wurtman, 1975) or PC (Magil et al., 1981), or consuming choline-rich foods (Cohen and Wurtman, 1976)

rapidly cause parallel changes in brain ACh levels; in the amounts of ACh released when neurons fire (Maire and Wurtman, 1985; Jackson et al., 1995); and in postsynaptic ACh-dependent functions like the control of rat striatal (Ulus and Wurtman, 1976) and adrenomedullary (Ulus et al., 1977a, b, c) tyrosine hydroxylase activities. The affinity of ChAT for its other substrate, acetyl-CoA—formed from glucose in mitochondria—is substantially greater ($K_m = 77$ μM; Rossier, 1977) than that for choline ($K_m = 540$ μM), however actual acetyl-CoA concentrations in the vicinity of ChAT may still be insufficient to saturate the enzyme, and thus might also affect the rate of ACh synthesis. In support of this possibility, administration of glucose has been found to stimulate ACh synthesis (Dolezal and Tucek, 1982), and to attenutate the depletion of brain ACh induced by giving a muscarinic antagonist (Ricny et al., 1992). In microdialysis studies, glucose enhanced the rise in ACh output produced by scopolamine (Ragozzino et al., 1994; Ragozzino and Gold, 1995). Systemic administration of glucose also increased hippocampal ACh release (Ragozzino et al., 1996, 1998; Kopf et al., 2001).

If choline levels in nerve terminals are reduced pharmacologically by administering a drug, HC3 that blocks the reuptake of free choline from the synapse, the synthesis and release of ACh also decline in parallel (Maire and Wurtman, 1985). Although such experiments confirm the importance of choline availability in controlling ACh synthesis, they do not necessarily allow it to be concluded that high-affinity choline uptake is the rate-limiting factor controlling intracellular choline levels or ACh biosynthesis. This synthesis is affected by any process that modifies the neuron's concentration of free choline, and these levels vary considerably as a function of plasma choline concentrations in addition, possibly, to changes in reuptake efficiency. Moreover, the choline that enters the neuron via high-affinity uptake apparently is not selectively used for acetylation as opposed to phosphorylation (Kessler and Marchbanks, 1979; Jope and Jenden, 1981). As discussed earlier, it is possible, but not yet clearly demonstrated, that the density or activity of high-affinity choline uptake sites in presynaptic membranes is affected by phosphorylation, neuronal firing, or the rate at which ACh is being released (Simon and Kuhar, 1975; Ferguson et al., 2003; Gates et al., 2004).

5.2 Biosynthesis of Choline-Containing Phosphatides

All cells use choline as an essential component of phospholipid subunits which, when aggregated, form all of their membranes. The principal subunit, the phosphatide PC, is synthesized from choline by the CDP-choline cycle (or "Kennedy Cycle"; Kennedy and Weiss, 1956) (*Figure 3.2-4*); PC, in turn, provides the phosphocholine moiety for the synthesis of SM, the other major choline-containing phospholipid (*Figure 3.2-6*).

The CDP-choline cycle involves three sequential enzymatic reactions (*Figure 3.2-4*): In the first (described earlier), catalyzed by CK, a monophosphate is transferred from ATP to the hydroxyl oxygen of the choline, yielding phosphocholine. The second, catalyzed by CTP:phosphocholine cytidylyltransferase (CT), transfers cytidylylmonophosphate (CMP) from CTP to the phosphorus of phosphocholine, yielding cytidylyldiphosphocholine (CDP-choline). The third and last reaction, catalyzed by CDP-choline:1,2-DAG choline phosphotransferase (CPT), bonds the phosphocholine of CDP-choline to the hydroxyl group on the 3-carbon of DAG, yielding the PC.

All these steps use compounds that the brain must obtain entirely or in part from the circulation, i.e., choline; a pyrimidine-like uridine for conversion to CTP; a poluunsaturated fatty acid-like DHA, and all three steps can also affect the overall rate of PC synthesis in brain (Cansev et al., 2005; Wurtman et al., 2006). Thus, choline administration increases brain phosphocholine levels in rats (Millington and Wurtman, 1982) and humans (Babb et al., 2004), because CK's K_m for choline (2.6 mM; Spanner and Ansell, 1979) is much higher than usual brain choline levels (35–60 μM). Most commonly, the second CT-catalyzed reaction is most rate limiting, either because not all of the CT is fully activated by being attached to a cellular membrane (Vance and Pelech, 1984) or because local CTP concentrations are insufficient to saturate the CT (Ross et al., 1997). As described later, when brain CTP levels are increased by giving animals uridine, CTP's circulating precursor in humans (Cansev et al., 2005), PC synthesis is accelerated.

The activity of CPT and the extent to which this enzyme is saturated with DAG can also control the overall rate of PC synthesis, for example, in PC12 cells extending neurites after exposure to the nerve growth factor (NGF) (Araki and Wurtman, 1997). If rodents are given a diet that contains both choline and uridine (as its monophosphate, UMP) and, by gavage, a PUFA (particularly the omega-3 fatty acids DHA or EPA), brain PC synthesis rapidly increases (Cansev et al., 2006; Wurtman et al., 2006), and absolute levels of PC per cell (DNA) or per mg protein increase substantially (e.g., by 40–50% after 4 weeks of daily treatment (Wurtman et al., 2006; *Table 3.2-5*). These treatments produce parallel or greater increases in each of the other membrane phosphatides, as well as in proteins localized within synaptic membranes, like

Table 3.2-5
Effects of UMP-supplemented diet and/or DHA on brain phospholipid levels in gerbils

Treatments	Total PL	PC	PE	SM	PS	PI
Control diet + Vehicle	403 ± 23	155 ± 8	69 ± 3	47 ± 3	34 ± 1	20 ± 2
One week						
UMP + DHA	436 ± 15	188 ± 8[a]	79 ± 6	57 ± 6	47 ± 1[c]	23 ± 1
Three weeks						
UMP + DHA	502 ± 12[c]	217 ± 5[c]	102 ± 4[c]	73 ± 5[b]	41 ± 1[a]	27 ± 1[a]
Four Weeks						
Control diet + Vehicle	351 ± 8	152 ± 6	65 ± 4	45 ± 2	33 ± 3	21± 2
UMP diet + Vehicle	367 ± 22	171 ± 8[a]	84 ± 8[a]	52 ± 5	35 ± 3	31± 2[b]
Control diet + DHA	392 ± 20	185 ± 12[a]	78 ± 5[a]	56 ± 3[a]	39 ± 3	32 ± 2[b]
UMP diet + DHA	442 ± 24[c]	220 ± 12[c]	113 ± 6[c]	73 ± 4[c]	46 ± 6[c]	36 ± 3[c]

Note: Groups of eight gerbils were given either a control diet and DHA's vehicle (5% gum Arabic solution, by gavage) or a UMP-containing (0.5%) diet and DHA (300 mg/kg; in 5% gum Arabic solution, by gavage) for 1 or 3 weeks. In another set of experiments, groups of eight gerbils were given either a control or a UMP-containing (0.5%) diet, and received orally (by gavage) DHA (300 mg/kg; in 5% gum Arabic solution) or just its vehicle for 4 weeks. At the end of each supplementation period, the gerbils' brains were harvested and assayed for phospholipids. Data are presented as nmol/mg protein. Data from 1-and 3-week-treated control diet and vehicle groups were pooled as there were no significant differences among these groups

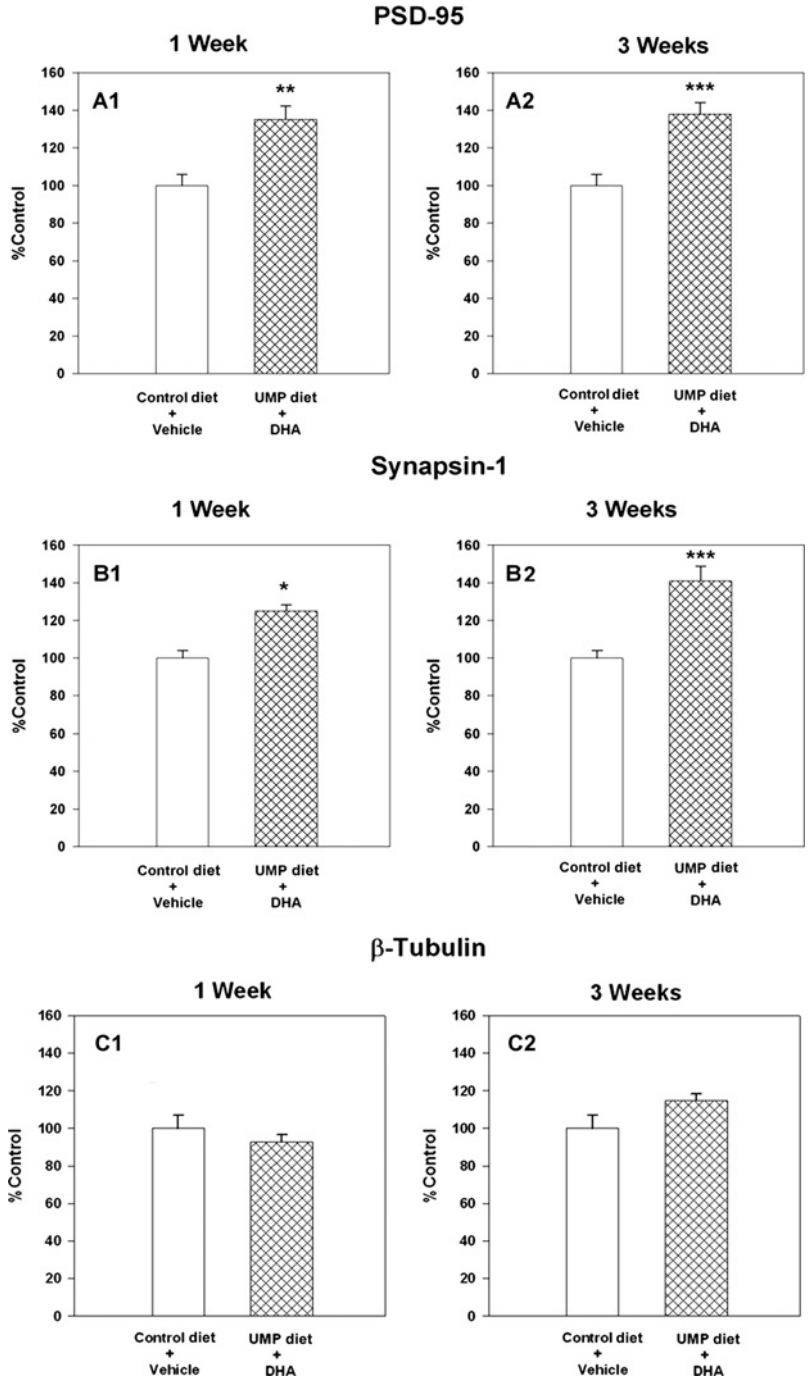
[a]$P<0.05$; [b]$P<0.01$; and [c]$P<0.001$ when compared with data from control diet and vehicle group

Abbreviations: Total PI, Total phospholipids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SM, sphingomyelin; PS, phosphatidylserine; PI, phosphatidylinositol (Reproduced from Wurtman et al., 2006)

synapsin-1, PSD-95 (*Figure 3.2-7*), and syntaxin-3 (Fujita and Kurachi, 2000; Ferreira and Rapoport, 2002; Darios and Davletov, 2006). They also increase formation of dendritic spines (Sakamoto and Wurtman, 2006) and the release of ACh (Wang et al., 2004) and dopamine (Wang et al., 2005a) from striatal neurons, and improve cognitive behaviors in aged rats (Teather and Wurtman, 2003), rats reared in a socially deprived environment, or normal animals (Teather and Wurtman, 2005). Hence, the production,

Figure 3.2-7
Increases in brain levels of PSD-95 and Synapsin-1 following dietary supplementation with uridine-5′-monophosphate (UMP) and docosahexaenoic acid (DHA). Groups of 8 gerbils were given either a control diet and DHA's vehicle (5% gum Arabic solution, by gavage) or a UMP-containing (0.5%) diet and DHA (300 mg/kg; in 5% gum Arabic solution, by gavage) for 1 or 3 weeks. At the end of each supplementation period, the gerbils' brains were harvested and assayed for PSD-95, Synapsin-1, and beta-tubulin. *$P<0.05$, **$P<0.01$, and ***$P<0.001$ when compared with data from control diet and vehicle group (Reproduced from Wurtman et al., 2006)

Figure 3.2-7 (continued)



levels, and functional properties of PC, and of other constituents of brain membranes, depend to a surprising extent on blood levels of its three circulating precursors.

DAG molecules are highly heterogeneous with reference to their fatty acid constituents; however, those containing DHA are preferentially used for phosphatide synthesis (Marszalek and Lodish, 2005; Marszalek et al., 2005). The incorporation of circulating DHA to form these molecules involves several steps: the fatty acid crosses the BBB (Hashimoto et al., 2002), then leaves the brain's extracellular space by partitioning into the external leaflet of a cell's plasma membrane. It then "flip-flops" to the inner leaflet, allowing it to interact with intracellular fatty acid-binding proteins and with the long-chain fatty acyl-CoA synthetase (LCFAS; EC 6.2.1.3) to form an acyl-fatty acid. This compound then attaches to the *sn*-2 position of glycerol-3-phosphate, a reaction catalyzed by acyl-CoA synthetase long-chain family member 6 (Acsl6; Marszalek et al., 2005). This product then attaches a saturated fatty acid at the *sn*-1 position to become phosphatidic acid, which is subsequently dephosphorylated to form DAG. The concentration of DHA in brain is only 1.2–2.9 μM (Deutsch et al., 1997; Contreras et al., 2000; Rosenberger et al., 2004) whereas the K_m of acyl-CoA synthetase is an order of magnitude higher (26 μM; Reddy et al., 1984). Hence, the enzyme is highly unsaturated with DHA, and very responsive to changes in DHA levels.

In vivo, orally administered DHA (200 mg/kg/day for 10 weeks to gerbils; Cao et al., 2006 or 300 mg/kg/day for 12 weeks to rats; Hashimoto et al., 2002, 2005) has been shown to elevate brain DHA levels by 10–25%; however, this effect was not observed in gerbils (Cao et al., 2005) or rats (Boswell et al., 1996) receiving 150 mg/kg/day or 1250 mg/kg/day, respectively, for 4 weeks, perhaps reflecting the slow mean turnover time for brain PC, and the speed with which DHA-containing phosphatides are deacylated and then reacylated with less-unsaturated fatty acids. In PC12 cells engineered to overexpress the Acsl6 protein, [^{14}C]DHA added to the medium is rapidly acylated to [^{14}C]DHA-CoA and incorporated into phospholipids and triglycerides (Marszalek et al., 2005; Richardson and Wurtman, 2006). That exogenous DHA increases brain PC and PE levels by increasing the phosphatide's synthesis is further supported by the finding that DHA administration concurrently raised PC and PE levels whereas lowering those of CDP-choline and CDP-ethanolamine (Wurtman et al., 2006), the immediate precursors for PC and PE, with which the DHA-containing DAG presumably combined. DHA and other PUFA can also promote membrane synthesis by an additional mechanism, i.e., by acting as agonists for a receptor, the syntaxin-3 protein (Darios and Davletov, 2006).

As with DHA, the mechanisms by which supplemental uridine increases phosphatide synthesis probably include two processes, increased substrate saturation of the enzymes that convert uridine, via uridine-5′-triphosphate (UTP) and CTP, to endogenous CDP-choline (Cansev et al., 2005), and activation by UTP of specific G-protein-coupled receptors, the P2Y receptors which affect neurite outgrowth (Pooler et al., 2005). A single dose of uridine, given as uridine-5′-monophosphate (UMP) to gerbils by gavage, has been shown to cause sequential increases in blood and brain uridine, then in brain UTP, CTP, and CDP-choline levels (Cansev et al., 2005). The uridine is carried across the BBB by a high-affinity concentrative nucleoside transport protein, CNT2, described later (Li et al., 2001; Gray et al., 2004); this protein's affinity for cytidine is only one-tenth or one-twentieth that for uridine (Larrayoz et al., 2006; Nagai et al., 2006a, b). Moreover, in contrast to uridine, little or no cytidine is present in human blood, any exogenous or endogenous circulating cytidine being rapidly converted to uridine in the human liver (Wurtman et al., 2000).

The effect of the uridine on phosphatide synthesis is short-lived, and insufficient in itself to produce reliable increases in brain PC or PE. However, chronic consumption of a uridine source (via the diet) for three or four weeks raises brain PC and PE levels substantially (❯ *Table 3.2-5*), even though the increases that this treatment produces in plasma uridine are small and transient. In vitro, uridine in concentrations of 50 μM or greater increases the size of neurites (Pooler et al., 2005) sprouting from NGF-stimulated PC12 cells (Araki and Wurtman, 1997); it also accelerates phosphatide synthesis (Richardson et al., 2003) and increases neurofilament protein levels in these cells (Pooler et al., 2005). The stimulatory effect of uridine on neuritogenesis also involves activation by UTP, its product, of P2Y receptors. This was shown by demonstrating that a lower concentration of UTP (10 μM) than that of free uridine can enhance neurite outgrowth (Pooler et al., 2005), and that the effects of both uridine and UTP on neuritogenesis are blocked by apyrase, a drug that degrades nucleotides, or by P2Y antagonists (Pooler et al., 2005).

Combined treatment of animals with dietary choline, UMP, and DHA tends to produce larger increases in levels of individual brain phosphatides (e.g., SM) than the sum of the increases caused by the individual precursors (*Table 3.2-5*). This probably reflects the operation of all the earlier mechanisms: Providing each of the three circulating precursors decreases the likelihood that its intracellular levels will become limiting when levels of the other precursors have been increased, and providing uridine or DHA enhances the activation of P2Y receptors by UTP, or of syntaxin-3 by the DHA. The relevant P2Y receptors might include any of the pyrimidine-sensitive receptors (P2Y2, P2Y4, or P2Y6) in the P2Y family.

Giving the three precursors concurrently increased PC by 21% (nmol/mg protein) after 1 week, and by 40% or 45% after 3 or 4 weeks; corresponding increases expressed as nmol/μg DNA were by 20%, 34%, and 41%, respectively (*Table 3.2-5*). Similar or greater changes are noted in the other phosphatides, or, in rat pups if the precursors were administered to their mothers during pregnancy and lactation (Marzloff et al., 2006). It is conceivable that even greater increases might be produced by administering larger doses of the precursors, or by extending the treatment for longer periods. The amounts of choline provided in the studies cited (Marzloff et al., 2006; Wurtman et al., 2006) were standard for gerbil and rat chows; UMP, the uridine source, is not normally included in such chows; however, it is present in human mother's milk and included in infant formulas in amounts intended to provide up to 0.3 mg/100 J (Yu, 2002); DHA is not a constituent of gerbil or rat chow, but is variably present in human diet depending in large part on the quantities of seafoods—particularly higher-fat fish like herring and salmon (Harris, 2005)—that a person elects to eat. Consumption of up to 3 g/day of DHA and other omega-3 fatty acids (e.g., eicosapentaenoic acid) is generally regarded as safe (GRAS) by the U. S. Food and Drug Administration, and doses of 4 g/day, or 50–60 mg/kg are sometimes used in the treatment of hypertriglyceridemia (Stalenhoef et al., 2000). The precise ratios of supplemental choline:uridine:DHA needed for optimal enhancement of brain phosphatide levels await determination, as does the question of whether AA or other PUFA present in phosphatides besides DHA should also be included. No data are presently available on possible behavioral consequences of increasing the amounts of synaptic membrane in human brain by administering DHA, a uridine source, and choline. Brain levels of choline, ethanolamine (Nitsch et al., 1992), and DHA (Soderberg et al., 1991) are all known to be subnormal among patients with Alzheimer's disease, and brains of such patients exhibit characteristic decreases in synaptic size and number (Terry et al., 1991; Selkoe, 2002; Coleman et al., 2004), as well as increases in the phosphatide breakdown products GPC and glycerophosphoethanolamine (Blusztajn et al., 1990; Nitsch et al., 1992). If brains of patients with Alzheimer's disease remain capable of responding to the three circulating phosphatide precursors by increasing brain levels of synaptic membrane, the precursors could conceivably confer some therapeutic benefit in this disease.

5.2.1 CTP:Phosphocholine Cytidylyltransferase

CTP:phosphocholine cytidylyltransferase (CT; EC 2.7.7.15) catalyzes the condensation of CTP and phosphocholine to form CDP-choline (*Figure 3.2-4*). CT is present in both soluble and particulate fractions of the cell (Wilgram and Kennedy, 1963); the cytosolic form is reportedly inactive whereas the membrane-bound form is active (Vance and Pelech, 1984; Tronchere et al., 1994). Increases in the association of CT with membranes reportedly correlate with increases in CT activity and in the net synthesis of PC in vitro (Sleight and Kent, 1980, 1983; Pelech et al., 1984). Some other lipids (reviewed in Cornell and Northwood, 2000) and DAG (Sleight and Kent, 1980; Utal et al., 1991) can stimulate the translocation of CT from the cytosol to membranes in vitro, thereby activating the enzyme. However translocation may not be the sole mechanism of CT activation, inasmuch as increases in the activity of membrane-bound CT do not always correlate with decreases in that of the cytosolic enzyme (Weinhold et al., 1991); as would be expected if traslocation were the only means whereby CT become activated. The phosphorylation state of CT may also be important (Watkins and Kent, 1991) as well as its substrate saturation with CTP and perhaps phosphocholine.

CT has been purified to homogeneity (Weinhold et al., 1986), and has been cloned from rat liver (Kalmar et al., 1990) and from a human erythroleukemic cell line (Kalmar et al., 1994). The purified form exists as an elongated dimer (Cornell, 1989). Mammalian CT proteins are divided into four functional domains: an N-terminal nuclear targeting sequence, a catalytic domain, a membrane–lipid binding domain, and a C-terminal phosphorylation domain. CT is termed CTα or CTβ depending on whether or not the N-terminal nuclear targeting domain does or does not contain a nuclear localization signal. The two genes that encode CT proteins reside on distinct chromosomes, i.e., *Pcyt1a*, which encodes the CTα isoform and is located on human chromosome 3q (Tang et al., 1997; Karim et al., 2003), and *Pcyt1b*, which encodes CTβ1, -β2, and –β3 isoforms and is X-linked (Lykidis et al., 1998, 1999; Karim et al., 2003).

The K_ms of CT for CTP and phosphocholine in brains of laboratory rodents and humans are 1–1.3 mM and 0.30–0.31 mM (Mages et al., 1988; Ross et al., 1997), respectively, whereas brain levels of these compounds are 70–110 μM (Mandel and Edel-Harth, 1966; Abe et al., 1987; Cansev et al., 2005) and 0.32–0.69 mM (Millington and Wurtman, 1982; Nitsch et al., 1992; Klein et al., 1993), respectively. Hence, brain CT is normally unsaturated with both of its substrates but especially CTP, suggesting a limiting role for cellular CTP in PC synthesis. In fact, treatments that increase cellular CTP levels do enhance the synthesis of CDP-choline and PC in poliovirus-infected HeLa cells (Choy et al., 1980); undifferentiated PC12 cells (Lopez G-Coviella and Wurtman, 1992; Richardson et al., 2003); rat striatal brain slices (Savci and Wurtman, 1995); and gerbil brain in vivo (Cansev et al., 2005).

5.2.2 CDP-Choline:1,2-Diacylglycerol Cholinephosphotransferase

CDP-choline:1,2-DAG cholinephosphotransferase (CPT; EC 2.7.8.2) catalyzes the final reaction in the Kennedy cycle; it transfers the phosphocholine moiety from CDP-choline to DAG, thus yielding PC and releasing CMP (❯ *Figure 3.2-4*). CPT, an integral membrane protein, is present primarily in the endoplasmic reticulum (Coleman and Bell, 1977). The enzyme protein has been solubilized and partially purified from microsomes of rat liver (Kanoh and Ohno, 1976; Ishidate et al., 1993), rat brain (Roberti et al., 1989), and hamster liver (O and Choy, 1990); purification to homogeneity has not been accomplished as the enzyme is membrane-bound, and thus is susceptible to detergents. A human cDNA has been isolated which codes for an enzyme with both cholinephosphotransferase and ethanolaminephosphotransferase (EPT) activities (hCEPT1; Henneberry and McMaster, 1999), and a different human cDNA has also been isolated which codes for an enzyme exhibiting only cholinephosphotransferase-specific activity (hCPT1; Henneberry et al., 2000). CPT may be a reversible enzyme, synthesizing CDP-choline from PC and CMP in liver (Kanoh and Ohno, 1973a, b) or brain microsomal preparations (Goracci et al., 1981, 1986; Roberti et al., 1992).

The CPT reaction is unsaturated with the enzyme's substrates; its K_m values for CDP-choline and DAG in rat liver are 200 μM and 150 μM (Cornell, 1992) respectively, whereas the concentrations of these compounds in liver are ~40 μM (Korniat and Beeler, 1975) and 300 μM (Turinsky et al., 1991), respectively (A DAG concentration of at least 1000 μM would probably be needed to stimulate the enzyme). Brain CDP-choline and DAG levels are even lower, i.e., about 10–30 μM (Alberghina et al., 1981; Cansev et al., 2005) and 75 μM (Abe et al., 1987), respectively. Levels of cellular DAG have been shown to limit PC synthesis in permeabilized HeLa cells (Lim et al., 1986), cultured rat hepatocytes (Jamil et al., 1992), and PC12 cells (Araki and Wurtman, 1997). In the latter study, cellular DAG levels had been increased by fivefold or none by exposing them to NGF. This treatment differentiated the cells, causing neurite outgrowth and a major increase in PC levels. None of these studies distinguished between the enzymes that act on both choline and ethanolamine (PECT1) and the enzyme that acts only on choline (PCT1). A more recent report, using cloning and expression methods, described that the K_m of human PECT1 for CDP-choline, as 36 μM (Wright and McMaster, 2002), which would probably still be too high to be saturated with this substrate in brain. The K_m of the enzyme for its substrates might also be affected by the fatty acid composition of the DAG molecule; for example, incubating mouse liver microsomes with DAG molecules that contained two oleic acids (1,2-dioleoyl-*sn*-glycerol; Di-$C_{18:1}$ (cis-9)) rather than two palmitic acids (1,2-dipalmitoyl-*sn*-glycerol [Di-$C_{16:0}$]), increased its K_ms for DAG from 86 ± 6 μM to 1860 ± 39 μM

and its K_m for CDP-choline from 41 ± 2 μM to 1000 ± 141 μM (Mantel et al., 1993). Hence the enzyme's affinity for its substrates declined by 20–25-fold.

5.2.3 Uptake of Uridine and Cytidine into Brain Cells

Uridine and cytidine are transported across cell membranes, including the BBB, through two families of transport proteins, i.e., the Na^+-independent, low-affinity, equilibrative transporters (ENT1 and ENT2) and the Na^+-dependent, high-affinity, concentrative (CNT1, CNT2, and CNT3) nucleoside transporters (Baldwin et al., 2004; Gray et al., 2004). The two ENT proteins, which transport uridine and cytidine with similar affinities, have been cloned from rat (Redzic et al., 2005) and mouse (Murakami et al., 2005) BBB. Inasmuch as their K_m values for the pyrimidines are in the high micromolar range (100–800 μM; Pastor-Anglada et al., 1998) they probably mediate BBB pyrimidine uptake only when plasma levels of uridine and cytidine have been elevated experimentally. In contrast, CNT2, which transports both uridine and purines like adenosine probably mediates uridine transport across the BBB under physiologic conditions. The K_m values for the binding of uridine and adenosine to this protein (which has been cloned from rat BBB; Li et al., 2001) are in the low micromolar range (9–40 μM in kidney, intestine, spleen, liver, macrophage, and monocytes; Griffith and Jarvis, 1996), whereas plasma uridine levels are subsaturating, i.e., 0.9–3.9 μM in rats (Traut, 1994); 3.1–4.9 μM in humans (Traut, 1994); and around 6.5 μM in gerbils (Cansev et al., 2005). Cytidine has not been thought to be a substrate for CNT2 (Gray et al., 2004); however, recent studies suggest that CNT2 can also transport it, but with a much lower affinity than that for uridine (Larrayoz et al., 2006; Nagai et al., 2006a, b).

Like other circulating compounds, pyrimidines may also be taken up into brain through the epithelium of the CP and the ENT1, ENT2, and CNT3 transporters (Baldwin et al., 2004; Gray et al., 2004); all these proteins have been found in CP epithelial cells of rats (Anderson et al., 1999a, b; Redzic et al., 2005) and rabbits (Wu et al., 1992, 1994). However, the surface area of BBB is probably 1000 times that of the CP epithelium (i.e., in humans 21.6 m^2 vs. 0.021 m^2; Pardridge, 2001); hence, in any event, the BBB is the major locus at which circulating uridine enters the brain. These more recent data on specific transport proteins confirm those from earlier studies on pyrimidine uptake across the BBB, which first demonstrated uridine's efficient (Hogans et al., 1971; Cornford and Oldendorf, 1975) and cytidine's poor (Galletti et al., 1991) uptake into brain. Moreover, cytidine is barely measurable in human plasma (Traut, 1994; Wurtman et al., 2000), and, as described later, when exogenous cytidine is given in the form of CDP-choline, it appears in human blood as uridine, not cytidine (Wurtman et al., 2000). Hence, uridine is the preferred circulating precursor for the CTP used in humans and some rodents (e.g., gerbils but not rats) for brain phosphatide synthesis (Cansev and Wurtman, 2005; Cansev, 2006).

5.2.4 Phosphorylation of Uridine and Cytidine to UTP and CTP

Uridine and cytidine are converted to their respective nucleotides following successive phosphorylations by various kinases (*Figure 3.2-4*). Uridine-cytidine kinase (UCK) (ATP:uridine-5′-phosphotransferase, EC 2.7.1.48), the first enzyme in this cascade, catalyzes the phosphorylations of uridine and cytidine to form UMP and cytidine-5′-monophosphate (CMP), respectively (Canellakis, 1957; Skold, 1960; Orengo, 1969). Several different forms of UCK exist, possibly as isoenzymes (Krystal and Webb, 1971; Absil et al., 1980). Humans have two such isoenzymes, UCK1 and UCK2, which have now been cloned (Koizumi et al., 2001; van Rompay et al., 2001).

The next enzyme in this sequence, which phosphorylates UMP and CMP to form uridine-5′-diphosphate (UDP) and CDP, respectively, is UMP–CMPK (ATP:CMP phosphotransferase, EC 2.7.4.14) (Hurwitz, 1959; Sugino et al., 1966; Ruffner and Anderson, 1969). UDP and CDP are further phosphorylated to UTP and CTP, by nucleoside diphosphate kinases (NDPK) (Nucleoside triphosphate:Nucleoside diphosphate phosphotransferase, EC 2.7.4.6) (Berg and Joklik, 1954; Parks and Agarwal, 1973). The

mRNAs for UCK1 (van Rompay et al., 2001) and UMP–CMPK (van Rompay et al., 1999) as well as NDPK activity have been described in brain (Langen et al., 1999; Kim et al., 2002).

The interconversions of uridine and cytidine, and of their respective nucleotides, are also observed in mammalian cells. Cytidine and CMP can be deaminated to uridine and UMP (Wang et al., 1950), whereas UTP is aminated to CTP by CTP synthase [UTP:ammonia ligase (ADP-forming), E.C. 6.3.4.2] (Lieberman, 1956; Hurlbert and Kammen, 1960). This enzyme acts by transferring an amide nitrogen from glutamine to the C-4 position of UTP, thus forming CTP (Zalkin, 1985). CTP synthase activity has been demonstrated in rat brain (Genchev and Mandel, 1974).

All the enzymes described earlier apparently are unsaturated with their respective nucleosides or nucleotides in brain and other tissues. For example, K_ms for uridine and cytidine of UCK prepared from various tissues varied between 33 and 270 μM (Skold, 1960; Orengo, 1969; Anderson, 1973; Greenberg et al., 1977), and the K_m for uridine of recombinant enzyme cloned from mouse brain was 40 μM (Ropp and Traut, 1996, 1998). Brain uridine and cytidine levels are about 22–46 pmol/mg wet weight (Mascia et al., 1999; Cansev et al., 2005) and 6–43 pmol/mg wet weight (Peters et al., 1987; Cansev et al., 2005), respectively. Hence, the syntheses of UTP and CTP, and the subsequent syntheses of brain PC and PE via the Kennedy pathway, depend on the availability of their pyrimidine substrates. Indeed, an increase in the supply of uridine or cytidine to neuronal cells, in vitro (Savci and Wurtman, 1995; Richardson et al., 2003; Pooler et al., 2005) or in vivo (Cansev et al., 2005; Cansev and Wurtman, 2005), enhanced the phosphorylation of uridine and cytidine, elevating the levels of UTP, CTP, and CDP-choline.

6 Physiological and Behavioral Effects of Choline

Administration of choline by direct placement into the CNS, orally or by injection, can produce numerous physiological or behavioral effects. Some of these are readily attributable to enhanced ACh release; others may be mediated by phospholipid metabolism or, conceivably, by direct actions of the choline on cholinergic receptors, as discussed earlier. Some remain unexplained.

6.1 Blood Pressure

Intravenous choline administration lowers blood pressure in both humans and animals (Mendel et al., 1912; Steigmann et al., 1952; Anton, 1954; Kapp et al., 1970; Singh, 1973; Savci et al., 2003). Intramuscular administration of choline (20 mg/kg/day for 3 days) to rats attenuates the fall in blood pressure induced by acute hemorrhage, and increases survival rate (Altura, 1978). Intraperitoneal choline (60 mg/kg) partially restores blood pressure after the induction of hypotension by acute hemorrhage (Ulus et al., 1995); in contrast intravenous choline (54 mg/kg) further decreases blood pressure and can cause death in hemorrhaged rats (Savci et al., 2003). Oral choline fails to affect cardiovascular function in rats (Ulus et al., 1979) but reportedly lowered blood pressure slightly in some patients with Alzheimer's disease (Boyd et al., 1977).

Intracerebroventricular choline (8 mg/dog) produced a biphasic blood pressure response in anesthetized dogs, an immediate and short-lasting (about 10–15 min) blood pressure rise (by 60–90 mm Hg) followed by a longer-lasting (about 60 min) fall (by 15–20 mm Hg; Srimal et al., 1969). In rats, intracisternal choline (12.5–50 μg; Kubo and Misu, 1981a) or its microinjection (1–3 μg/site; Kubo and Misu, 1981b) into the dorsal medulla lowered blood pressure, whereas intracerebroventicular choline (50–150 μg/rat) raised blood pressure and decreased heart rate (Caputi and Brezenoff, 1980; Arslan et al., 1991; Isbil-Buyukcoskun et al., 2001; Li and Buccafusco, 2004) for 5–20 min. In rats, intracerebreventricular choline (25–150 μg/rat) restored normal blood pressure among animals made hypotensive by acute hemorrhage (Ulus et al., 1995; Savci et al., 2002b), endotoxin (Savci and Ulus, 1997), chemical sympathectomy (Gurun et al., 1997a), spinal cord transection (Savci and Ulus, 1998), autonomic ganglion blockade, or α-adrenoceptor blockade (Savci and Ulus, 1996). At a dose of 180 nmol, choline also potentiated the pressor responses evoked by naloxone or glycyl-glutamine (Gurun et al., 2003). In normotensive rats, the blood pressure responses to choline administered centrally involve local activation of both mAChRs and nAChRs (Arslan et al., 1991);

including the α7 nAChR subtype (Li and Buccafusco, 2004). In hypotensive animals, these blood pressure responses involve presynaptic activation of central nAChRs (Ulus et al., 1995; Savci and Ulus, 1996, 1997, 1998; Gurun et al., 1997a; Savci et al., 2002b).

6.2 Body Temperature

Intracerebroventricular (75–300 μg/rat), but not intraperitoneal (30–120 mg/kg), choline decreases body temperature (Unal et al., 1998). This hypothermia is mainly mediated by M1and M3 muscarinic receptors (Unal et al., 1998).

6.3 Pain

Choline can alter responses to painful stimuli in experimental animals, and can modify the actions of analgesic drugs. Given intraperitoneally (15–60 mg/kg) to rats, it diminishes the analgesic actions of morphine (10 mg/kg; subcutaneously) as assessed using the hot-plate test (Botticelli et al., 1977). In mice, subcutaneous (up to 500 mg/kg; Damaj et al., 2000) or intravenous (2–64 mg/kg; Wang et al., 2005b) choline fails to alter responses to thermal pain, but when given intracerebroventricularly (Damaj et al., 2000; Wang et al., 2005b) or intrathecally (Damaj et al., 2000) at doses of 30–120 μg/animal, it produces antinociception in the same pain model. This latter effect can be blocked by the nonselective mAChRs antagonist atropine or by antagonists of α7 nAChRs (e.g., metillycaconitine; α-bungarotoxin). Intravenous choline (4–64 mg/kg) produced significant antinociception in mice in the late phase of inflammatory pain responses to subcutaneous injection of 5% formalin (Wang et al., 2005b). At a 2 mg/kg dose, it also enhanced the antinociceptive effects of aspirin (9.4 mg/kg; i.v.) and morphine (0.165 mg/kg; i.v.). This action was also blocked by the α7 nAChR antagonists metillycaconitine and α-bungarotoxin, but not by atropine or naloxone (Wang et al., 2005b).

6.4 Neuroendocrine Effects

Choline produces a variety of neuroendocrine responses when administered peripherally or centrally to rats. Its oral administration (20 mmol/kg by stomach tube) for 4 days increased urinary catecholamine output (Scally et al., 1978). Given intraperitoneally, choline (30–120 mg/kg) elevated plasma catecholamines (Ilcol et al., 2002c) and insulin (Ilcol et al., 2003a). Given intracerebroventricularly (50–150 mg/rat), it increased plasma concentrations of the catecholamines (Arslan et al., 1991; Ulus et al., 1995; Gurun et al., 2002); vasopressin (Arslan et al., 1991; Ulus et al., 1995; Savci and Ulus, 1996, 1998; Gurun et al., 1997a; Savci et al., 2003); ACTH (Savci et al., 1996); β−endorphin (Savci et al., 1996); and prolactin (Gurun et al., 1997b). The increases in plasma vasopressin, ACTH, and β-endorphin were found to be mediated by central nAChRs, and that of prolactin by mAChRs (Gurun et al., 1997b). The increases in plasma insulin after intraperitoneal choline involve both ganglionic nAChRs and the M1 and M3 subtypes of mAChRs (Ilcol et al., 2003a). Choline's site of action in producing rest of these neuroendocrine effects is principally presynaptic, i.e., via enhancing ACh release (Scally et al., 1978; Savci et al., 1996, 1998, 2003; Ilcol et al., 2003a), and the effects are enhanced by hemorrhagic, hypotensive, or osmotic stresses (Scally et al., 1978; Ulus et al., 1995; Savci et al., 1996, 1998, 2003; Ilcol et al., 2003a).

6.5 Peripheral Metabolism

Peripheral (40–120 mg/kg; intraperitoneal) or central (75–300 μg/rat; icv) administration of choline increases blood glucose levels in rats (Gurun et al., 2002; Ilcol et al., 2002c). This hyperglycemic response is prevented by blockade of ganglionic nAChRs or α-adrenoceptors, as well as by bilateral adrenalectomy

(Ilcol et al., 2002c), and apparently is mediated by the stimulation of adrenomedullary catecholamine release and subsequent activation of α-adrenoceptors. It apparently also involves activation of central nAChRs affecting the sympathoadrenal system (Gurun et al., 2002). As decribed earlier, glucose is a source of acetyl-CoA, which, like choline, is a limiting precursor for ACh. Hence, choline administration may enhance ACh synthesis by this additional mechanism.

6.6 Behavior

Acute administration of choline to humans, as choline chloride or as PC, improved short-term memory in some studies (Leathwood et al., 1982; Ladd et al., 1993) but not in others (Davis et al., 1980; Harris et al., 1983). Intraperitoneal choline (6–60 mg/kg) in combination with glucose (10–30 mg/kg) improved passive avoidance behavior in mice (Kopf et al., 2001). Chronic consumption of a choline-rich diet by aged mice reportedly counteracted the age-associated decline in learning and memory (Bartus et al., 1980; Golczewski et al., 1982; Leathwood et al., 1982).

Supplementation of pregnant or lactating rats with choline chloride during the perinatal period (embryonic days 12–17 and/or postnatally 16–30 days) caused long-lasting improvements in spatial memory, as illustrated using radial-arm maze tests (Meck et al., 1988, 1989; Meck and Williams, 1997b, 1999; Williams et al., 1998) and the Morris water maze test (Schenk and Brandner, 1995; Tees, 1999a, b; Tees and Mohammadi, 1999). In adulthood, rats that had received supplemental choline (about four times dietary levels) perinatally exhibited increased memory capacity (Meck and Williams, 1997c) and precision (Meck and Williams, 1999), and performed more accurately on tests of spatial memory (Meck et al., 1988, 1989; Meck and Williams, 1997a, b, c, 1999; Williams et al., 1998). Moreover, perinatal supplementation with choline provided some protection against memory impairments usually associated with normal aging (Tees and Mohammadi, 1999), neonatal alcohol exposure (Thomas et al., 2004; Wagner and Hunt, 2006), or epileptic seizures (Yang et al., 2000; Holmes et al., 2002).

Although the mechanism by which perinatal choline supplementation produces these long-term effects on memory remains unclear, such supplementation is known also to cause enduring neuroanatomic and neurochemical changes in brain regions involved in memory. For example, choline supplementation increased hippocampal ChAT activity, mAChRs density (Meck et al., 1989), ACh release (Cermak et al., 1998), and the amplitude of ACh-mediated excitatory potentials (Montoya et al., 2000), and it reduced acetylcholinesterase activity (Cermak et al., 1998, 1999). Moreover, basal forebrain cholinergic neurons projecting to the hippocampus were larger and more spherical among rats that had been supplemented perinatally with choline (Loy et al., 1991; Williams et al., 1998). Prenatal choline supplementation also reportedly enhanced MAPK and CREB activation (Mellott et al., 2004); N-methyl-D-aspartate (NMDA) receptor-mediated neurotransmission (Montoya and Swartzwelder, 2000); long-term potentiation (LPT) (Pyapali et al., 1998); PLD activity (Holler et al., 1996); NGF levels (Sandstrom et al., 2002); and dendritic spine formation (Mervis, 1982) in rat hippocampus. It also decreased the rate of apoptosis in the hippocampus and basal forebrain of 18-day old fetuses (Holmes-McNary et al., 1997) and increased brain cell division (Albright et al., 1999a, b). Prenatal supplementation with choline also was associated with greater excitatory responsiveness, reduced slow afterhyperpolarizations, enhanced afterdepolarizing potentials, larger somata, and greater basal dendritic arborization in hippocampal CA1 pyramidal cells studied postnatally at 20–25 days of age (Li et al., 2003).

6.7 Drug Interactions

Acute or chronic choline administration can modify the actions of some centrally active drugs. Chronic (28–35 days) treatment of rats with choline through the diet (about ten times more choline than in control diets) produced behavioral hyperactivity and attenuated the sedative/hypnotic and hypotermic effects of pentobarbital (Wecker et al., 1987). Chronic choline supplementation also increased the density of binding sites for nicotine (Coutcher et al., 1992) and α-bungarotoxin (Morley and Garner, 1986) and produced

tolerance to the convulsive and lethal actions of nicotine (Wecker et al., 1982). Chronic dietary choline supplementation to mice modulated benzodiazepine receptor binding and γ-aminobutyric acid receptor function (Miller et al., 1989), also decreasing seizure activity and the lethality of such seizure-promoting drugs such as nicotine, paraoxon, strychnine, and pentylenetetrazol (Wecker et al., 1982). Acute intraperitoneal choline administration (100 mg/kg) to morphine-dependent rats decreased withdrawal symptoms and the associated weight loss (Pinsky et al., 1973; Frederickson and Pinsky, 1975).

6.8 Neuroprotective and Cytoprotective Effects

Choline exhibits cytoprotective and neuroprotective actions in vivo and in vitro: In vitro, choline itself or some of its analogs (i.e., mono-, di-, and triethylcholine and pyrrolidinecholine), can, at 1–10 mM concentrations, protect against manifestations of cytotoxicity in differentiated PC12 cells induced by growth factor deprivation (Jonnala et al., 2003). Choline (5–75 mM) suppresses the development of dark cell degeneration in Purkinje neurons following receptor activation with AMPA (DL-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid; Strahlendorf et al., 2001). At concentrations of 1 mM or greater it also reduces NMDA toxicity in organotypic hippocampal slice cultures derived from neonatal rats (Mulholland et al., 2004), and reportedly it is cytoprotective in PC-12 cells (Jonnala et al., 2003); Purkinje neurons (Strahlendorf et al., 2001); and hippocampal cells (Mulholland et al., 2004)—an effect mediated by the α7 subtype of nAChRs. Prenatal choline supplementation for 6 days during days E12–E17 of gestation protected against subsequent neurodegeneration in the posterior cingulate and retrosplenial cortices, induced in female adolescent rats by peripheral administration of dizocilpine (Guo-Ross et al., 2002).

In cultured neonatal cardiac ventricular cells, choline (0.1–1 mM) reduced H_2O_2-induced apoptotic cell death, by acting via M3 mAChRs (Yang et al., 2005). In vivo, choline (20 mg/kg; i.v.) attenuated endotoxin-induced multiple organ injury (i.e., renal, hepatic, and cardiac injuries) in dogs (Ilcol et al., 2005b). Similarly, in rats, choline supplementation in the diet for 3 days attenuated endotoxin-induced hepatic injury and improved survival (Rivera et al., 1998). Choline-induced protection from the tissue injuries induced by endotoxin is associated with a reduction in serum levels of tumor necrosis factor-α (TNF-α) (Rivera et al., 1998; Ilcol et al., 2005b) and with the improvement in platelet counts and platelet closure times (Yilmaz et al., 2006a). Intravenous choline administration (5 mg/kg) protected rats (5 mg/kg; i.v.) from ischemic myocardial injuries by stimulating M3-mAChRs (Yang et al., 2005).

7 Effects of Exogenous CDP-Choline

Cytidine-5′-diphosphocholine (CDP-choline; citicoline), which is composed of choline and cytidine linked by a diphosphate bridge, is both an essential intermediate in the synthesis of endogenous PC through the Kennedy cycle (*Figure 3.2-4*), and a drug used in some countries to treat cerebral ischemia, traumatic brain injury, Parkinson's disease, or stroke.

When administered orally or parenterally, exogenous CDP-choline is completely hydrolyzed, first to cytidine monophosphate and phosphocholine, and then to free cytidine and choline (Lopez G-Coviella et al., 1987). In humans, the cytidine is further transformed to uridine, hence giving CDP-choline to humans causes dose-related increases in serum uridine and choline levels but not in serum cytidine (Wurtman et al., 2000; Cansev, 2006). In laboratory rodents, depending on species and on the activity of the hepatic enzyme cytidine deaminase (Chabot et al., 1983; Kühn et al., 1993), which converts cytidine to uridine, CDP-choline administration can principally elevate either serum uridine (e.g., in gerbils) or cytidine (e.g., in rats), besides choline. Moreover, as treatments that elevate plasma uridine (or cytidine) and choline thereby increase brain PC synthesis (Wurtman et al., 2000; Richardson et al., 2003; Cansev et al., 2005; Ulus et al., 2006) as well as steady-state brain levels of PC and other membrane phosphatides (Lopez G-Coviella et al., 1992, 1995; Wurtman et al., 2006), it is likely that some of CDP-choline's therapeutic actions result from changes that it produces in the quantities or the composition of brain membranes. Some other effects of exogenous CDP-choline (Savci et al., 2002a, 2003; Cavun and Savci, 2004;

Cavun et al., 2004) probably are mediated by increasing ACh release, secondary to the rise it produces in plasma and brain choline levels (Savci et al., 2002a, 2003).

By increasing brain levels of endogenous CDP-choline, exogenous CDP-choline also increases the amounts of DAG that combine with this intermediate to form PC (and that combine with endogenous CDP-ethanolamine to form PE) (Araki and Wurtman, 1998). CDP-choline administration can also affect the brain by increasing the amount of free AA that is used to form DAG, which then combines with endogenous brain CDP-choline. This causes a decrease in free AA levels, which might otherwise be neurotoxic, and thus it decreases the ultimate size of the brain damage following a stroke and brain injury (Warach et al., 2000). This reduction in AA may be the major mechanism underlying CDP-choline's acute therapeutic effects (Lopez G-Coviella et al., 1998). It has also been suggested that exogenous CDP-choline may decrease brain levels of free AA by directly inhibiting phospholipase A2 activity or by decreasing the formation of that enzyme protein (Adibhatla and Hatcher, 2003; Adibhatla et al., 2006).

7.1 Hypoxia and Ischemia

Intracerebral CDP-choline (0.6 μmoles) prevented the ischemia-induced loss of radioactive choline from glycerophospholipids, and suppressed the increase in brain levels of free fatty acids in a global model of ischemia in rats (Dorman et al., 1983; Goldberg et al., 1985). In a rat model of transient cerebral ischemia, intraperitoneal CDP-choline (250 mg/kg; twice a day for 4 days) improved neurological signs and attenuated the increases in glucose and pyruvate levels and the decrease in ACh synthesis from labeled glucose (Kakihana et al., 1988). In an ischemic and anoxic rat model, CDP-choline (300 mg/kg, i.p.) decreased the incidence of neurological deficits (Yamamoto et al., 1990). In a chronic hypoxia rat model produced by placing animals in chambers in which the oxygen content was depressed (7–15%) for extended time periods, CDP-choline (100 mg/kg in food) protected vigilance behavior (Hamdorf and Cervos-Navarro, 1990), reduced hypoxia-induced behavioral deterioration (Hamdorf and Cervos-Navarro, 1991), and increased survival time at 7% O_2 (Hamdorf and Cervos-Navarro, 1991; Hamdorf et al., 1992). In a model of rat experimental hypoxia induced by giving potassium cyanide, oral CDP-choline given for 4 days before the induction of hypoxia increased survival time (Tornos et al., 1983a). Araki et al. (1988) also observed a neuroprotective effect of CDP-choline in mice in which the cerebral ischemia was induced by decapitation or by potassium cyanide intoxication. CDP-choline (500 mg/kg; i.p.) for 14 days delayed cell membrane damage and behavioral dysfunction in spontaneously hypertensive rats in which ischemia had been caused by artificially induced occlusion of the lateral middle cerebral artery (Aronowski et al., 1996). In a similar study, CDP-choline (500 mg/kg; i.p.) decreased infarct volume and edema in a rat model of temporary focal ischemia (Schabitz et al., 1996). In mice with an intracerebral hemorrhage, CDP-choline (500 mg/kg; i.p.) reduced the volume of ischemic injury surrounding the hematoma, and improved the behavioral outcome (Clark et al., 1998). In another study, CDP-choline (400 mg/kg; i.p.) increased blood pressure, reduced infarct volume, and decreased the mortality rates of hypotensive rats with a experimental subarachnoid hemorrhage (Alkan et al., 2001). In rats with permanent occlusion of the middle cerebral artery, CDP-choline inhibited MAP kinase signaling pathways (Krupinski et al., 2005). In a focal brain ischemia model in rats, CDP-choline (0.5–2 g/kg; i.p.) reduced infarct size and inhibited ischemia-induced decreases in cortical and striatal ATP levels (Hurtado et al., 2005).

CDP-choline produces synergistic neuroprotective effects when this treatment is combined with glutamate receptor antagonists (i.e., MK-801; Onal et al., 1997 or lamotrigine; Ataus et al., 2004); thrombolytic agents (i.e., recombinant tPA; Andersen et al., 1999; De Lecinana et al., 2006 or urokinase; Shuaib et al., 2000); the calcium channel blocker, nimodipine (Sobrado et al., 2003); or basic fibroblast growth factor (Schabitz et al., 1999) using experimental ischemia models in rats.

In a gerbil model in which brain ischemia was produced by bilateral ligation of the carotid arteries, intraventricular (0.6 mmol; Trovarelli et al., 1981) or intraperitoneal (150 mg/kg; Trovarelli et al., 1982) CDP-choline partially prevented the ischemia-induced increases in fatty acids and decreases in PC levels (Trovarelli et al., 1981, 1982). CDP-choline reduced the dysfunctions of the BBB after reperfusion in gerbils (Rao et al., 1999), and reduced the cerebral edema, concurrently reducing the levations of AA levels and

leukotriene C4 synthesis (Rao et al., 2000). In a transient cerebral ischemia model, CDP-choline (500 mg/kg daily for 2 days; i.p.) restored the decreases in PC, SM, cardiolipin, and total glutathione levels induced by ischemia (Adibhatla et al., 2001).

In cats undergoing brief periods of cerebral ischemia, CDP-choline attenuated the depression in the cortical evoked potentials (Boismare et al., 1978).

7.2 Head Trauma (Cranio-Cervical Trauma)

Neuroprotective effects of CDP-choline have been described in studies using various trauma models and experimental animals. In a weight-drop concussive head injury model in mice, CDP-choline (60–250 mg/kg) shortened the recovery time (Boismare et al., 1977). In a controlled lateral-impact model in rat, CDP-choline (100 mg/kg; i.p.) increased extracellular ACh levels, decreased cognitive deficits and attenuated the trauma-induced increased sensitivity to the memory-disrupting effects of scopolamine (Dixon et al., 1997). In a cortical impact model in rat, intraperitoneal CDP-choline (400 mg/kg) reduced brain edema (Baskaya et al., 2000), and decreased neuronal loss in the hippocampus, and improved neurological recovery (Dempsey and Raghavendra Rao, 2003). In a rat experimental (weight-drop) spinal cord injury model, CDP-choline (400 mg/kg; i.p.) improved behavioral and neuroanatomic signs of recovery (Yucel et al., 2006).

7.3 Induced Lesions

Neuroprotective actions of CDP-choline have also been demonstrated in lesion studies. Oral administration of CDP-choline, at a daily dose of 1 g/kg for 4 days, significantly extended survival time and increased the percentage of survivors from KCN-induced toxicity (Tornos et al., 1983a). CDP-choline administration (500 mg/kg, i.p.) for 7 days ameliorated functional behavior, as shown by reducing the number of apomorphine-induced contralateral rotations. It also attenuated the loss of substantia nigra dopaminergic neurons and the decrease in tyrosine hydroxylase immunoreactivity, in the ipsilateral striatum in rats injected intrastriatally with the dopaminergic toxin, 6-hydroxydopamine (Barrachina et al., 2003). CDP-choline (62.5–250 mg/kg, i.p.) protected hippocampal neurons against apoptosis and the degeneration induced by injecting beta-amyloid into brains of rats also undergoing cerebral hypoperfusion (Alvarez et al., 1999). CDP-choline (50 mg/kg) prevented mice and rats from an acrylamide-induced neurological syndrome (Agut et al., 1983). In tissue culture studies, CDP-choline protected the retinal ganglion cells (Oshitari et al., 2002) and prevented glutamate-mediated cell death in cerebellar granule neurons (Mir et al., 2003).

7.4 Other Effects

Oral or intraperitoneal administration of CDP-choline (10–500 mg/kg, for 5–7 days) improved memory in rats with memory deficits induced by muscarinic AChR antagonists, by the α2-adrenoceptor agonist clonidine, by electroconvulsive shock, or by hypoxia (Petkov et al., 1992, 1993). Dietary CDP-choline supplementation protected rats against the development of memory deficits in aging (Teather and Wurtman, 2003), and prevented memory impairments caused by impoverished environmental conditions (Teather and Wurtman, 2005). In humans, CDP-choline improved verbal memory in aging (Spiers et al., 1996) and benefitted memory in elderly subjects (Alvarez et al., 1997).

In rat striatum, CDP-choline activated tyrosine hydroxylase (Martinet et al., 1981), increased dopamine levels (Martinet et al., 1979; Shibuya et al., 1981), and enhanced K^+-evoked dopamine release (Agut et al., 2000) and haloperidol-induced elevation in dopamine metobolites (Agut et al., 1984). Oral CDP-choline increased the total urinary excretion of the noradrenaline metabolite 3-methoxy-4-hydroxyphenylglycol, in rats and humans (Lopez G-Coviella et al., 1986). Centrally administered CDP-choline (0.5–2.0 μmol)

increased plasma vasopressin (Cavun et al., 2004), and ACTH concentrations and potentiated the release of GH, TSH, and LH (Cavun and Savci, 2004) stimulated by clonidine, TRH, and LHRH, respectively. Intravenously injected CDP-choline (250 mg/kg) increased plasma concentrations of noradrenaline and adrenaline in rats (Savci et al., 2003).

Oral administration of a single dose (2 g/kg) of CDP-choline to mice decreased the intensity of the morphine withdrawal syndrome (Tornos et al., 1983b). In a single study, treatment with CDP-choline was reported to affect some measures of craving in cocaine-dependent human subjects (Renshaw et al., 1999).

CDP-Choline increased blood pressure, reversed hypotension in hemorrhagic shock (Savci et al., 2002a, 2003), and prolonged survival time (Yilmaz et al., 2006b) when given intravenously (100–500 mg/kg) or intracerebroventricularly (0.1–2.0 μmol) to rats.

CDP-choline decreased platelet reactivity to aggregating agents when given acutely (250 mg/kg) and increased the antiaggregatory activity of aortic walls when given chronically (250 mg/kg, i.p., 2 weeks) to rats. In dogs, CDP-choline (70 mg/kg; i.v.) prevented the endotoxin-induced decrease in circulating platelet counts and prolonged platelet closure times (Yilmaz et al., 2006a).

Intracerebroventicular CDP-choline (0.5–2.0 μmol) produced antinociception in three different acute pain models (i.e., thermal paw withdrawal tests, mechanical paw pressure test, and acetic acid writhing test) in rats (Hamurtekin and Gurun, 2006).

7.5 Clinical Studies

CDP-choline effects have been examined in studies involving numerous normal subjects and patients with cerebral ischemia, traumatic brain injury, hypoxia, Alzheimer's and Parkinson's diseases. To date sufficient evidence has not been accumulated regarding any such use to warrant its approval for drug status by the US Food and Drug Administration. It is, however, approved for sale in a few other countries and sold under its international nonproprietary name, citicoline.

Clinical trials conducted in the USA, tested daily oral doses of 500, 1000, or 2000 mg/day, given for 6 weeks. In some such studies, the drug was administered within the first 48 h of an ischemic stroke (Clark et al., 1999); in others, it was first given to patients up to 14 days after the onset of the ischemic episode (Tazaki et al., 1988). Pooling of individual patients data from four USA trials yielded evidence that CDP-choline treatment could improve overall recovery at 12 weeks in acute ischemic stroke patients (Davalos et al., 2002). Pooled diffusion-weighted magnetic resonance imaging data from two clinical trials showed a significant dose-dependent reduction on percent change in lesion volume (Warach, 2002).

8 Choline in Autonomic and Motor Neurons

All nerves that leave the brain or spinal cord (i.e., axons of motor neurons, parasympathetic preganglionic neurons, and sympathetic preganglionic neurons), as well as all postganglionic parasympathetic neurons, release ACh as their neurotransmitter, and in all of them choline availability determines the rates at which ACh is synthesized and released. ACh is also present in the periphery in placenta, lymphocytes, the bladder, and tracheal epithelium; however, in these cells the effects of increasing choline availability on ACh synthesis have not yet been determined.

More than 50 years ago, Hutter (1952) demonstrated that a low intravenous dose (7 mg/kg) of choline enhanced neuromuscular transmission, whereas a high dose (50 mg/kg) blocked this transmission. Hutter also demonstrated that choline, at doses of 3–60 mg/kg, could restore neuromuscular transmission in curarized cats. Based on these observations, he suggested that choline increased ACh output from motor nerve endings. More recently, using the isolated, vascularly perfused rat phrenic nerve-hemidiaphragm preparation Bierkamper and Goldberg (1979, 1980) directly demonstrated that choline (at 30–60 μM concentrations) could increase ACh release at the neuromuscular junction.

Effects of choline on ACh synthesis and release, and on cholinergic neurotransmission, at parasympathetic synapses have been demonstrated in vivo (Kuntscherova, 1972; Haubrich et al., 1974, 1975;

Ilcol et al., 2003a) and in vitro, using isolated hearts (Dieterich et al., 1978), and atrial (Meyer and Baker, 1986) and pancreatic minces (Ilcol et al., 2003a). Choline infusion (10 μM) increases by 2–3-fold ACh release evoked by electrical stimulation of the vagus nerve in chicken hearts, and by at least 23-fold in cat heart (Dieterich et al., 1978). The presence of choline (10 μM) in the perfusion medium also increased, by—two to threefold, ACh release evoked by electrical field stimulation (at 20 Hz for 20 min) from isolated chicken, rat, cat, and guinea pig hearts (Dieterich et al., 1978). Subcutaneous choline administration (200 mg/kg) increased, by 34%, the ACh content of the atrium (Kuntscherova, 1972) and in atrial minces, choline (at 1–100 μM) increased ACh synthesis and release in a concentration-dependent manner (Meyer and Baker, 1986). In rats, intraperitoneal choline (90 mg/kg) increased, by 45%, the ACh contents of pancreatic tissue; this was associated with increased cholinergic neurotransmission to insulin secreting β-cells (Ilcol et al., 2003a). Choline (10–130 μM) also increased ACh synthesis and release from rat pancreatic minces (Ilcol et al., 2003a).

Oral choline administration (20 mmol/kg) to rats increases adrenal ACh levels by more than twofold for 8 h (Ulus et al., 1977a) and tyrosine hydroxylase activity by about 30% (Ulus et al., 1977a). Repeated oral administration of choline for 4 days increases the enzyme activity further, by up to 50–60%; the increase in tyrosine hydroxylase activity is not observed after intubation with saline, water, or ammonium chloride, and fails to occur in rats pretreated with cycloheximide (Ulus et al., 1977a). Similar increases in tyrosine hydroxylase activity after oral choline administration are observed in sympathetic ganglia, including the superior cervical ganglion, the stellate and celiac ganglia (Ulus et al., 1977c, 1979; Ulus and Wurtman, 1979), and the ganglia of the thoracic sympathetic chain (Ulus et al., 1977c). These increases in tyrosine hydroxylase activity are also not seen in adrenals after adrenal denervation (Ulus et al., 1977a) or after decentralization of superior cervical ganglion (Ulus et al., 1979), indicating that the action of choline is transsynaptic and that it requires intact preganglionic cholinergic nerves to affect ACh synthesis and release. Further evidence that choline administration enhances ACh release was obtained by studies in which choline was administered along with reserpine, or with other drugs that increase impulse flow in preganglionic cholinergic nerves. Injection of reserpine (2.5 mg/kg; i.p.) phenoxybenzamine (20 mg/kg; i.p.), or insulin (2 units/rat; i.p.) daily for 4 days, or of 6-hydroxydopamine (200 mg/kg twice, with an interval of 48 h, through tail vein), all caused marked increases in adrenal tyrosine hydroxylase activities. When these treatments were combined with oral choline (2.8 g/kg; by stomach tube), the resulting increases in tyrosine hydroxylase activity were considerably greater than the sum of the changes produced by choline alone and drug alone, that is, significant potentiation occurred (Ulus et al., 1977a, b, 1978). Potentiation of a treatment-induced rise in tyrosine hydroxylase by choline was also observed in the adrenals of rats kept in the cold (Ulus et al., 1977a, 1978) and in superior cervical ganglia of reserpine-treated rats (Ulus et al., 1977a). Taken together, these data indicate that the availability of free choline is a major factor controlling cholinergic neurotransmission in the sympathoadrenal system.

Studies using the classical perfused-superior cervical ganglion system failed to demonstrate parallel increases in ACh release in response to elevating the choline concentration of the perfusion media (Birks and MacIntosh, 1961; Matthews, 1966; MacIntosh, 1979; O'Regan and Collier, 1981). However, when superior cervical ganglia were perfused with choline-containing (10–14 μM) plasma or with Locke solution, they released greater amounts of ACh, by twofold, during a 1-h stimulation (20 Hz) period than ganglia superfused without exogenous choline (Birks and MacIntosh, 1961; Matthews, 1966; MacIntosh, 1979). Furthermore, the ACh stores in perfused-ganglia stimulated without exogenous choline were found to be partly depleted, although these ganglia had managed to synthesize some ACh by reusing free choline generated from the hydrolysis of released ACh, or from hydrolyzing membrane phospholipids like PC). PC levels and the number of synaptic vesicles in the cat's superior cervical ganglion were found to decline significantly after stimulation of the preganglionic nerve trunk if the uptake of exogenous choline was blocked by HC-3 (Parducz et al., 1976), or if the ganglia were perfused with a choline-free Locke solution (Parducz et al., 1986). In striking contrast, stimulated ganglia supplied with exogenous choline maintained their ACh stores (Birks and MacIntosh, 1961; Matthews, 1966; MacIntosh, 1979), as well as membrane PC levels and the numbers of storage vesicles (Parducz et al., 1976, 1986), although they released much more ACh than they initially contained.

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