Amino Acids (1998) 14:271–300

Amino
Acids

A review of physiological and metabolic effects of essential amino acids

Review Article

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Accepted June 30, 1997

Summary. The authors review ten essential amino acids with regard to their metabolic, physiologic and therapeutic effects throughout the human body. Physical properties of these biologically active compounds are discussed as a foundation for their diverse roles in special nitrogen containing products, neurotransmitters, and as alternative energy sources. Both normal and abnormal amino acid metabolism are considered in the areas of digestion, elimination of metabolic products, metabolic intermediates, and defects in these systems. Recent developments in therapeutic applications are further examined for clinical utility and as an economical alternative to traditional clinical treatment modalities.

Keywords: Essential amino acids – Therapeutic applications – Physiological effects –Metabolism

Introduction

Although over 300 amino acids have been described in nature, only twenty of these are typically found as components in human peptides. Of the twenty coded for by human DNA, ten are considered essential since human metabolic pathways are insufficient to synthesize them at adequate rates from other precursors. The essential amino acids are arginine, histidine, methionine, threonine, valine, isoleucine, phenylalanine, tryptophan, leucine, and lysine. However, arginine and histidine are essential only during periods of high anabolic activity, such as tissue growth during childhood. Although amino acid function is usually linked to peptide structure and function, peptides are not the major emphasis of this paper. The purpose of this review is to survey the essential amino acids by describing their physical characteristics and metabolic significance before providing an overview of their therapeutic application.

The essential amino acids have a few common structural features. Each has a carboxyl group and an amine group attached to an alpha carbon.

Various side chains, also attached to the alpha carbon, gives the different essential amino acids their characteristic physical and metabolic properties. At a physiological pH of 7.4, the carboxyl group is a carboxylate anion due to dissociation of the hydroxy proton, while the amino group takes on an additional proton and forms a positive charge. Some of the side chains are charged at physiological pH (i.e. 7.4), and play a critical role in determining the function of a particular amino acid in a peptide since the alpha amino and carboxyl groups are occupied in the peptide bond (Voet and Voet, 1990). However, with free amino acids, no peptide bond exists and the alpha carboxyl and amino species are charged (Martin et al., 1983). The nature of the side chain allows some amino acids to have unique roles, such as the physiological buffer formed by the histidine component of hemoglobin.

Free essential amino acids play a significant role in both primary metabolism and in the formation of specialized nitrogen (N) containing products. Some contribute to intermediates of the Glycolytic and Krebs (Tricarboxylic Acid Cycle) cycles. Hence, amino acids are used as a source of energy when carbohydrate or fatty acid levels have been depleted (Newsholme and Blomstrand, 1996). Some tissues, such as muscle, have the capacity to directly catabolize the branched chain essential amino acids valine, leucine, and isoleucine (Voet and Voet, 1990). Others play a part in neurotransmitter formation and can alter significant physiological characteristics such as mood. Tryptophan, for example, is a precursor of the neurotransmitter 5-hydroxytryptamine (serotonin) (Gowenlock, 1988). Methionine is an example of an essential amino acid that has a significant role in formation of special products requiring single carbon transfers. Modifications occur to methionine which produce a high energy S-adenosylmethionine product. This metabolite has an important role in the formation of choline, a significant part of many other high-energy metabolic intermediates (Zeisel and Blusztajn, 1994). There is a tremendous amount of literature dealing with the physiological significance of free amino acids and their effects on a variety of metabolic and physiological systems. Their importance is often illustrated when a nutritional deficiency occurs or an important enzyme is defective.

Deficiencies in essential amino acid metabolism give rise to a plethora of pathological conditions. The absence of an essential amino acid in the diet or a genetic defect in a key enzyme can render other metabolites essential, such as phenylalanine (Martin et al., 1983). Tyrosine is not essential so long as an adequate supply of both phenylalanine and the necessary enzyme to convert phenylalanine to tyrosine is present (Bhagavan, 1992). However, tyrosine becomes an essential amino acid when a deficiency or genetic defect occurs in this system. Some enzymatic deficiencies are benign, but others are debilitating and life-threatening. An understanding of the pathways of essential amino acid metabolism is vital for effective treatment of genetic defects altering these reactions. These defects provide insight into the important roles essential amino acids have in human metabolism.

Amino acids and their metabolic and physiological ramifications are among the most investigated topics in biomedical science. Many current stud-

ies focus on the therapeutic applications of exogenous amino acids. In this review of essential amino acid metabolism and therapeutic modalities, the focus will be to describe the effects of exogenous amino acid administration on each physiological system. Although amino acids most importantly give rise to complex peptides such as immunoglobulins, carrier proteins, and neurotransmitters, they also have unique biological and metabolic qualities as free amino acids. This review summarizes the ten essential amino acids along with a relevant overview of amino acid metabolism and therapeutic implications.

Overview of the physical properties of the essential amino acids

Amino acids, as the building blocks of the most diverse biological compounds, have a characteristic structure. With the exception of proline, all twenty amino acids have an amino group and a carboxyl group with a functional group covalently bound to the alpha carbon. In the essential amino acids, the functional groups are used to classify the amino acids into polar, non-polar, or basic side chains. See Table 1 for reference. The chemical nature of the side chain found on a particular amino acid plays a very important role in determining where the amino acid is found in the tertiary structure of a peptide chain.

There are several ways to classify amino acids, the most prevalent being the chemical nature of the side chains. Most essential amino acids have nonpolar functional groups. These include valine, leucine, isoleucine,

Table 1. Physical and metabolic properties of essential amino acids

Amino acid	Metabolic category	R Group category	pK1*	pK2**	pK3***
arginine	glucogenic	basic	2.2	9.0	12.5
histidine	glucogenic	basic	1.8	9.2	6.0
methionine	glucogenic	nonpolar	2.3	9.6	–
threonine	glucogenic	uncharged polar	2.2	9.1	10.1
valine	glucogenic	nonpolar	2.3	9.6	–
isoleucine	glucogenic and ketogenic	nonpolar	2.3	9.6	–
phenylalanine	glucogenic and ketohenic	nonpolar	2.3	9.6	–
tryptophan	glucogenic and ketogenic	nonpolar	2.3	9.6	–
leucine	ketogenic	nonpolar	2.3	9.6	–
lysine	ketogenic	basic	2.2	9.0	12.5

*This is the dissociation constant of the carboxyl group attached to the alpha-amino carbon; ** this is the dissociation constant of the amino group attached to the alpha-amino carbon; *** this is the dissociation constant of the titratable group in the side chain.

phenylalanine, tryptophan, and methionine. The side chains in this category do not accept or donate protons (H+) at physiological pH. Also, there is no participation in hydrogen bonding. Amino acids with nonpolar side chains tend to be located on the interior of protein molecules where they interact with other non polar (aliphatic) amino acids, and are shielded from polar entities, including water (Bhagavan, 1992). One exception to this is proteins located in lipid rich environments like cellular membranes, where a reverse arrangement occurs and the nonpolar amino acids are located peripherally to the peptide (Bhagavan, 1992).

Threonine has an uncharged polar side chain, which is neutral (uncharged) at the physiological pH of 7.4. Threonine contains an uncharged polar hydroxyl group and is capable of hydrogen bonding, which contributes to the tertiary structure of peptides. The hydroxyl substituent can serve as an important site of attachment for other molecules during biochemical reactions. Threonine can undergo a dehydration reaction with an oligosaccharide to form glycoproteins. The specific three-dimensional location of threonine in a peptide is a determining factor as to whether or not the hydroxyl group will be glycosylated. This type of reaction usually occurs as a peptide is being transferred from the endoplasmic reticulum to the Golgi apparatus (Newsholme and Leech, 1983).

Histidine, lysine and arginine are classified according to their basic side chains. By convention, these amino acids are proton acceptors at physiological pH (7.4). At this pH of 7.4, lysine and arginine are protonated and positively charged. Histidine, however, has a pKa of 6.0 for the imino nitrogen and is weakly basic. As a result, histidine in primarily in an uncharged state as a free amino acid at physiological pH (7.4) (Orten and Neuhaus, 1975). Histidine may have either a positive or neutral charge depending upon the microenvironment of the component peptide it is a part of.

The pKa of amino acid side chains is a reliable predictor of which will be an effective physiological buffer. Usually, a weak acid and its conjugate base will effectively buffer a solution within ± 1 pH unit of its pKa. For histidine, the pKa of the imidazole nitrogen in the side chain is approximately 6.0 (Orten and Neuhaus, 1975). When the pH is lower than the pKa, the protonated form is most abundant, and when pH is above pKa, the deprotonated form predominates. This physical quality enables histidine to be a significant physiological buffer. Although the most effective pH at which histidine can buffer a solution corresponds to the pKa, 6.0 in this case, the concentration of the acid and conjugate base are important as well. The more concentrated they are the greater the "buffering capacity".

Side chain characteristics, the ionic form present at a particular pH, and the acid/base qualities of free amino acids help develop a picture of how these biological molecules function in their diverse roles. The nature of the histidine side chain explains how plasma pH is protected from dangerous fluctuations. Reactivity of the hydroxyl component of the threonine side chain illustrates that when oligosaccharides are added in a hydrolysis reaction, the result is the formation of glycoproteins (Martin et al., 1983).

Metabolism of essential amino acids

The purpose of this section is to provide a general overview of the basic processes that allow the essential amino acids to participate in intermediate metabolism as well as form special products.

Nutritional considerations

Essential amino acids must be obtained from the diet. A controversy currently exists as to the appropriate dietary quantity for each of the essential amino acids. The debate is partly due to inadequate previous technology for measuring amino acid requirements, such as nitrogen balance and growth (Zello et al., 1995). Most estimates of essential amino acid requirements, based on nitrogen balance appear too low (Zello et al., 1995; McLarney et al., 1996), with the exception of the sulfur-containing methionine (Young, 1994). Other techniques used to estimate amino acid requirements are: plasma amino acid concentration, direct amino acid oxidation, and indicator amino acid oxidation (Zello et al., 1995). Protein metabolism in the body causes the elimination of nitrogen by urea production, which is the quantitatively most important catabolite. The conversion of the remaining amino acid carbon skeletons to intermediates of energy-producing pathways and other intrinsic metabolic consumption creates a steady need for dietary essential amino acids. The minimal nutritional requirement for humans and other mammals is heavily effected by age, stage of growth, and metabolic state. For example, children and patients in a catabolic state (i.e. burn, surgery) have a greater basal metabolic requirement for the essential amino acids than does a healthy adult (McLarney et al., 1996). Although controversy exists regarding the amounts of dietary intake of the essential amino acids, this review will deal only with the observation that these amino acids must be obtained from dietary sources in order to have normal growth, development, and maintenance.

Digestion

In the American diet, the typical consumption of dietary amino acids is approximately 100 g/day (Newsholme and Leech, 1983). These amino acids initially enter the body in the form of proteins and are subsequently hydrolyzed by chemical reagents and enzymes. Most of the proteolytic enzymes responsible for degrading dietary protein come from the stomach, pancreas, and small intestine (Berne and Levy, 1993).

Dietary protein digestion begins in the stomach as a result of gastric juice secretion. Gastric juice contains both hydrochloric acid and pepsin. Although the stomach pH is not sufficiently acidic to hydrolyze most peptides, it does denature them, making enzymes more efficient at hydrolyzing the peptide bonds. Pepsin is secreted as the proenzyme pepsinogen. Upon exposure to the acidic environment in the stomach, pepsinogen is converted to the active form, and is referred to as pepsin. Pepsin is unique in that other active pepsin

molecules can autocatalytically activate pepsinogen as well. Pepsin cleavage of peptides releases a few free amino acids and smaller peptides which then enter the duodenum of the small intestine (Newsholme and Leech, 1983).

Pancreatic proteases act in the small intestine to further degrade the peptides produced by the action of pepsin in the stomach. The pancreatic proteases are also released as inactive proenzymes which are all activated by the active form of trypsin, which is converted from the inactive form, trypsinogen, by a brush border enzyme called enteropeptidase (Berne and Levy, 1993). All the pancreatic proteolytic enzymes have specificity regarding the peptide bonds they cleave (Berne and Levy, 1993). Details about the specific enzymes and amino acids cleaved are not essential to this overview of digestion. However, the end result is the production of oligopeptides (smaller peptides) and additional free amino acids.

The final stage of peptide digestion involves aminopeptidase, an enzyme located at the luminal border of the small intestine. This enzyme repeatedly cleaves the N-terminal amino acid of the oligopeptides to produce free amino acids and some di-peptides (Berne and Levy, 1993). Although both the free amino acids and dipeptides are absorbed into the epithelial cells of the small intestine, only free amino acids are found in the portal system. While in the cytosol of the intestinal epithelial cells the dipeptides are hydrolyzed to free amino acids. After release into the portal system, the free amino acids are either metabolized by the liver or released into the general circulation (Berne and Levy, 1993).

Removal of nitrogen and excretion

Since there is no storage form of dietary nitrogen (N), any nitrogen not immediately required for the metabolic processes must be eliminated from the body. Transamination and oxidative deamination reactions are responsible for the initial step of amino acid catabolism. The alpha-amino N is removed by either of these processes (Torchinsky, 1987). With transamination, there is the production of an alpha-ketoacid and glutamate. Of the essential amino acids, only lysine does not undergo a transamination (Newsholme and Leech, 1983). The alpha-ketoacids may participate in the synthesis of other non-essential amino acids, while the glutamate formed can be deaminated to form alpha-ketoglutarate. Alpha-ketoglutarate is the receptor of the alpha-amino group in transamination reactions (Torchinsky, 1987).

Oxidative deamination results in the direct elimination of the alpha-amino group as ammonia. The ammonia produced in this reaction acts as a source of nitrogen in urea production. None of the essential amino acids undergo rapid oxidative deamination. However, the glutamate formed by transamination is converted to ammonia by the glutamate dehydrogenase enzyme. This indirect mechanism provides a method for converting the amino group of most amino acids to ammonia. In this way, glutamate acts as a funnel by which many amino acids can be converted to intermediates of the TCA cycle (Martin et al., 1983).

The ammonia produced by oxidative deamination provides a source of nitrogen in the Urea Cycle, which is the major pathway for elimination of amino acid nitrogen. Production of urea occurs in the liver and is excreted in the urine (Martin et al., 1983).

Conversion to primary metabolic intermediates

Once released into the general circulation, amino acids are transported into cells by an active transport process, which is coupled to the cleavage of ATP. With the exception of the branched chain amino acids, most carbon skeleton metabolism occurs in the liver (Newsholme and Leech, 1983). After the alpha-amino groups have been removed from the free amino acids, the remaining carbon skeletons are available for conversion to one of seven intermediates involved in the primary energy pathways. These intermediates are oxaloacetate, alpha-ketoglutarate, pyruvate, fumarate, acetyl CoA, succinyl CoA, and acetoacetate (Voet and Voet, 1990). Metabolism of the carbon skeleton after transamination leads to the production of ketogenic or glucogenic intermediates, or both intermediates (Martin et al., 1983).

Leucine and lysine are exclusively ketogenic. All ketogenic amino acids must converge to form acetoacetate, acetyl CoA, or acetoacetyl CoA. Through a series of conversions, leucine and lysine form acetyl CoA which is then converted to a molecule of acetyl CoA. The acetyl CoA can enter the TCA cycle at the point were pyruvate is normally split into two molecules of acetyl CoA.

Four of the essential amino acids are exclusively glucogenic in their catabolism; arginine, histidine, methionine, and valine (Voet and Voet, 1990). These are called glucogenic due to the fact that they are catabolized to intermediates of glucose production, which occurs in the liver. All glucogenic amino acids form either pyruvate or an intermediate of the TCA Cycle and can participate in gluconeogenesis (formation of glucose) or glycogenesis (formation of glycogen) in both the liver and muscle. Arginine and histidine form alpha-ketoglutarate of the TCA Cycle. Methionine, threonine and valine enter the TCA Cycle as succinyl CoA. In the catabolism of arginine it is cleaved by the enzyme arginase to produce ornithine, which is then transaminated to yield glutamate-gamma-semialdehyde, which is then converted to alpha-ketoglutarate via glutamate. Although threonine may be converted to propionyl CoA, the precursor of succinyl CoA, it is also capable of direct conversion to pyruvate which subsequently enters the TCA Cycle. In both cases, threonine is still considered a glucogenic essential amino acid (Voet and Voet, 1990).

Four essential amino acids are both glucogenic and ketogenic. They are isoleucine, phenylalanine, threonine, and tryptophan (Voet and Voet, 1990). Catabolism of these essential amino acids yields both the ketogenic precursors that contribute to fatty acid synthesis, and the glucogenic metabolites of the Krebs cycle. The catabolism of isoleucine yields acetyl CoA and succinyl CoA (Voet and Voet, 1990). Tryptophan catabolism involves a substantial

sequence of reactions that eventually lead to the formation of acetoacetyl CoA. Phenylalanine metabolism leads to the production of fumaryl-acetoacetate which is cleaved to produce fumarate and acetoacetate (Newsholme and Leech, 1983).

Metabolic defects in essential amino acid metabolism

Mutant genes resulting in a abnormal enzymes are the most common cause of amino acid metabolic errors. Although a few inborn deficiencies are benign, others lead to profound developmental problems which can be life threatening. When a given pathway is blocked by a defective enzyme, the metabolites prior to that step accumulate to levels that can cause anything from darkening of urine to mental retardation and even death. At least fifty disorders have been described regarding enzymatic deficiencies in amino acid metabolism. Although the frequency of any one disorder is not very high (1:10,000 for phenylketonuria) they collectively form a family of genetic metabolic disorders that are commonly treated by pediatricians (Bonon and Rosenberg, 1980; Eisensmith and Woo, 1994).

One of the more common defects in essential amino acid metabolism involves a defect in the enzyme phenylalanine hydroxylase, which manifests itself in the condition called phenylketonuria (PKU) (Guttler and Guldberg, 1994). This enzyme normally converts phenylalanine to tyrosine. When defective, phenylalanine levels are elevated in plasma, tissues and urine. Derivatives of phenylalanine, not normally produced in significant amounts, are significantly elevated in PKU patients. Infants with PKU are born with a normal IQ and without CNS damage since the excess phenylalanine is cleared across the placenta. Upon constant exposure to elevated levels of phenylalanine, neurophysiological deficits invariably occur (Griffiths et al., 1995). Infants with PKU do not typically exhibit elevated levels of phenylalanine until approximately 24 hours after protein ingestion. The symptoms associated with PKU include mental retardation, microencephaly, growth inhibition, seizures, and impaired motor skills (Diamond et al., 1994). An additional characteristic of PKU subjects is a lack of pigmentation due to the high levels of phenylalanine which competitively inhibit formation of tyrosine hydroxylase, the first step in melanin formation.

During phenylalanine catabolism, a deficiency of homogentisate oxidase leads to alcaptonuria, which is a benign condition in which homogentisate accumulates in the urine (Hegedus and Nayak, 1994). The excess homogentisate forms polymers that cause the urine to darken upon standing.

Elevated levels of phenylalanine can also be attributed to deficiencies in enzymes that form tetrahydrobiopterin, which phenylalanine hydroxylase requires as a coenzyme (Mitnaui and Shiman, 1985). When phenylalanine hydroxylase is inhibited by a genetic flaw, tyrosine becomes essential as well, and has to be supplemented in a special diet.

Three common enzyme deficiencies are associated with the conversion of methionine to succinyl CoA. Homocystinuria is related to a deficiency of cystathione synthetase where homocysteine accumulates in the

urine (Sperandeo et al., 1995). Methionine, S-adenosylmethionine, and S-adenosylhomocysteine are elevated in blood plasma. The systemic effects of Homocystinurea are neurological deficits in the brain (Brouwer, 1992), decalcification of bone (osteoporosis) (Tsuda and Sakiyama, 1990), a displacement of the lens in the eye (Juszko et al., 1994), and it is associated with premature development of vascular disease (Boers, 1994). Cystathionurea is due to a deficit of cystathionase which is asymptomatic. However, cystathione and its metabolites do accumulate. Propionyl carboxylase deficiency affects the conversion of propionyl CoA to the Kreb's intermediate, succinyl CoA (Lamhonwah et al., 1990). This defect has an impact on the metabolism of valine, isoleucine and methionine because it occurs after they converge to form propionyl CoA. As with most carboxylase enzymes, propionyl carboxylase contain biotin. A deficiency of propionyl carboxylase leads to elevated plasma levels of propionate and accumulation of odd numbered fatty acids in the liver. Developmental problems are associated with this defect as well.

Another common genetic defect is histidemia. It has a prevalence of 1:10,000 live births, and is due to a deficiency of the histidase enzyme. In subjects with this enzyme deficiency, histidine accumulates in the urine and plasma. Although not always present, mental retardation can occur (Virmani and Widhalm, 1993).

Glutaric Acidemia type 1 is a rare autosomal recessive inborn error of lysine and tryptophan metabolism. The deficient mitochondrial enzyme is glutaryl coenzyme A dehydrogenase, resulting in episodic elevations in glutaric acid (Yanicelli et al., 1994). Neurological damage and eventually death may ensue in untreated patients. Treatment consists of a lysine and tryptophan restricted diet with oral administration of riboflavin and L-carnitine. Glutaric acidemia is most successfully treated if therapy is initiated before neurological damage occurs.

When an error in the metabolism of branched-chain amino acids (BCAA) occurs, the result is an accumulation of valine, leucine, isoleucine and their metabolic intermediates. Normally, it is the excess of the metabolic intermediates which cause the most serious biological effects. The most common disorder of BCAA metabolism is maple syrup urine disease, caused by an inherited deficiency of branched-chain alpha-ketoacid dehydrogenase (Mamer and Reimer, 1992; Mogos et al., 1994). This disease is usually characterized by sweet smelling urine, seizures, coma, physical and mental retardation. However, there can be defects in other areas of the metabolism of BCAA. Generally, these lead to ketoacidosis which is usually treatable (Ko et al., 1991; Sovik, 1993).

Special products and systemic effects of essential amino acid metabolism

Aside from their important roles in enzymes and other proteins, essential amino acids are also precursors for many physiologically significant nitrogen-containing molecules. Among the special products are; neurotransmitters, hormones, and nucleotide bases. This section describes briefly the roles of

essential amino acids in the formation of these and other physiologically significant products.

Arginine

During catabolism, arginine is cleaved by arginase to produce ornithine which is then transaminated to yield glutamate semialdehyde and is subsequently converted to alpha-ketoglutarate via glutamate. Arginine can be converted to creatine when combined with glycine and a methyl group from S-adenosylmethionine. Creatine is reversibly phosphorylated to creatine phosphate, which is a high energy derivative that reversibly donates a phosphate group to ADP in the formation of ATP. This reaction provides a small but rapidly mobilized energy reserve in muscle (Orten and Neuhaus, 1975). In the liver arginine serves an important role in the excretion of nitrogen containing compounds. As an intermediate in the urea cycle, arginine is cleaved into ornithine and urea. The urea is excreted in the urine while the ornithine re-enters the urea cycle (Martin et al., 1983). The arginine is the primary component in the synthesis of nitric oxide (NO) (Ganong, 1993). Nitric oxide is synthesized from arginine, molecular oxygen, and NADPH by the enzyme nitric oxide synthase. FMN, FAD, heme, and tetrahydrobiopterin are all necessary coenzymes for this reaction.

Arginine, through nitric oxide (NO), is a mediator in a variety of biological systems. Since nitric oxide is functionally identical to endothelium-derived relaxing factor, it causes vasodilation by relaxing vascular smooth muscle (Laurant and Demolombe, 1995; Nakaki and Kato, 1994; Turner et al., 1996). Studies with mineralocorticoid-salt hypertensive rats and corticotropin-induced high blood pressure have found that L-arginine has an antihypertensive effect. The mechanisms of action for arginine are through the vascular effects of NO and the reduction of serum corticosterone concentrations respectively (Laurant and Demolombe, 1995; Turner et al., 1996). In addition, arginine's inhibition of the renin-angiotensin system helps to cause a decrease in the systolic blood pressure (Higashi et al., 1995).

In a study of healthy men it was found that oral administration of L-arginine prevented platelet aggregation. Its inhibition was directly correlated to plasma levels of arginine,while heart rate and fasting lipid levels were unaffected (Adams et al., 1995). Another effect of arginine, D- and L-stereoisomers, is the production of hormonal responses (Davies et al., 1995). Systemic infusion of arginine increases the plasma insulin, glucagon, and prolactin levels (MacAllister et al., 1995). Also, arginine, through nitric oxide (NO), stimulates the release of dopamine from the striatum in gerbils (Strasser et al., 1994), increases the tumoricidal and bactericidal actions of macrophages (DiLorenzo et al., 1995), stimulates wound healing (Sac, 1994), and has neurotransmitter functions in the brain (Lefebvre, 1995).

Tryptophan

Tryptophan is converted into two products: niacin and the neurotransmitter, serotonin (Shibata, 1995). Serotonin has many diverse physiological functions

such as pain perception (Haze, 1991; Russel et al., 1992), affective disorders, and sleep (Imeri et al., 1994), temperature (Schwartz et al., 1995), and blood pressure (Samoil and Grubb, 1995). In the formation of serotonin, tryptophan is converted to 5-hydroxytryptophan in which tetrahydrobiopterin and molecular oxygen are necessary for this reaction. 5-hydroxytryptophan is then decarboxylated to serotonin. Although niacin is readily available from lean meat, cereals and enriched grains, it can also be synthesized inefficiently from tryptophan (Shibata, 1995). The metabolic pathway producing niacin from tryptophan only produces 1 mg of nicotinic acid for every 60 mg of tryptophan consumed. The production of niacin is only functional when a relative overabundance of the amino acid is available. This in vivo inefficiency renders dietary niacin as the primary source (Shibata, 1995).

An increase in tryptophan levels leads to an increased concentration of 5-hydroxytryptamine (serotonin) in the brain. This effect can be observed in neurons which are related to sleep function (Imeri et al., 1994). An excess of serotonin in brain tissue is known to increase the mental effort necessary to maintain physical exercise (Newsholme and Blomstrand, 1996; Davis, 1995). Acute ethanol consumption is known to decrease circulating tryptophan availability (Badawy, 1995). One of the major systemic effects of tryptophan is through its 5-hydroxytryptamine metabolite (Newsholme and Leech, 1983). Serotonin has been implicated in various psychiatric disorders (Lucca et al., 1995). Tryptophan depletion has been used successfully to evaluate central serotonin levels in depression and other neuropsychiatric disorders. Tryptophan depletion reduces brain serotonin function, which in turn reduces the therapeutic effects of specific serotonin reuptake inhibitors, but not drugs that inhibit noradrenaline reuptake. Rather than having a neurotransmitter function, tryptamine has been suggested to play a neuromodulatory role. A decrease in erythrocyte tryptophan uptake in schizophrenic patients is correlated with a loss of impulse control (Serres et al., 1995). The decrease in tryptophan uptake is a reflection of peripheral tryptophan metabolism. The majority of scientific literature indicates that 5-hydroxytryptamine acts directly as a neurotransmitter.

Tryptophan has been implicated as a possible explanation of central fatigue (Newsholme and Blomstrand, 1996; Davis, 1995). During extended exercise free tryptophan levels are elevated and, therefore, serotonin (Newsholme and Blomstrand, 1996). This increases activity in the brain of neurons that can induce sleep.

Phenylalanine

Pietz et al. found that when PKU patients were administered a bolus of 100 mg/kg phenylalanine, both the plasma and brain concentrations of phenylalanine increased significantly with no impairment of attentional and fine motor scores on neurophysiological tests for up to 20 hours postload (Pietz et al., 1995). However, most of the systemic effects of phenylalanine are a result of the enzyme phenylalanine hydroxylase converting phenylalanine to

tyrosine (Zello et al., 1990). Thus, this nonessential amino acid, tyrosine, overlaps substantially with phenylalanine. L-tyrosine is ultimately converted to fumarate and acetoacetate intermediates which join primary metabolic pathways as both glucogenic and ketogenic precursors (Bhagavan, 1992). Phenylalanine and tyrosine form the initial two biosynthetic steps of dopamine formation (Lou, 1994). The rate limiting enzyme that catalyzes dopa, the immediate precursor to dopamine, is tyrosine hydroxylase (Fitzpatrick, 1991). This enzyme is abundant in the adrenal medulla, sympathetic nervous ganglia, and in the central nervous system (Haycock, 1991; Lewis et al., 1993). Dopamine serves as a neurotransmitter in the brain whose importance is illustrated by Parkinson's Disease where nuclei in the subthalmic region fail to produce adequate quantities of this neurotransmitter (Hirsch, 1994). Deficiency of phenylalanine causes a diminished synthesis of dopamine which has adverse effects on performance of mental tasks (Zello et al., 1990).

Dopamine, a precursor of epinephrine and norepinephrine, when hydroxylated forms norepinephrine (Milner and Wurtman, 1986). Norepinephrine is a catecholamine that is released as a neurotransmitter from postganglionic sympathetic nerves and also functions as a hormone, along with epinephrine, when released by the adrenal medulla. S-adenosylmethionine, a metabolite of the essential amino acid methionine, donates a methyl group to norepinephrine to form epinephrine (i.e. adrenalin) (Kennedy et al., 1990).

Histidine

Histidase deaminates histidine to form N-formiminoglutamate (FIGlu), which transfers the formimino substituent to tetrahydrofolate and forms glutamate (Martin et al., 1983). A dietary deficiency of folic acid results in increased urinary elimination of FIGlu and is the basis of the FIGlu excretion test (Armstrong et al., 1991; Koblin et al., 1990). Tetrahydrofolic acid (THF), a metabolite of histidine and the active form of folic acid, serves an important role in one carbon metabolism. Dihydrofolate reductase converts the inactive folic acid to its active form with the addition of two protons (H+) and two molecules of NADPH. The various forms of THF are essential in methionine, thymidine, purine-C8, and purine-C2 synthesis. Unlike humans, some microorganinsms synthesize folic acid directly. In humans, methotrexate inhibits the conversion of folic acid to its active form and is a useful regimen for patients with acute lymphocytic leukemia (Fleisher, 1993; Gokbuget and Hoelzer, 1996).

Histidine has effects on a variety of organisms and physiological systems. As a free radical scavenger, histidine quenches hydroxyl and hydrogen peroxide, but not superoxide anions (Cai et al., 1995). The total free energy of cells are increased as the adenine nucleotide pool is elevated following histidine administration (Cai et al., 1995).

Histidine plays an important role in the modulation of oxidative DNA degradation. Protonation of the imidazole component of histidine abolishes

the capacity of histidine to modulate the oxidative degradation of DNA (Ouzou et al., 1994). Evidence suggests that at low hydrogen peroxide levels, the protective effect of histidine may be a result of its ability to bind hydroxy free radicals. Histidine also reduces ischemia induced by myocardial injury in isolated perfused rat hearts (Cai et al., 1995).

An important metabolite of histidine is the chemical messenger histamine. Histidine is decarboxylated to form this powerful component of many allergic and inflammatory reactions. Histamine mediates a variety of cellular actions such as gastric acid secretion (Pattichis and Louca, 1995), vasodilation (Laight et al., 1995), and allergic reactions (Okahara et al., 1995). Histamine has also been suggested as a possible neurotransmitter in the brain (Okahara et al., 1995). Although histamine is not used clinically, histamine antagonists have important therapeutic functions.

Branched chain amino acids (valine, leucine, isoleucine)

The branched chain essential amino acids (BCAA) have similar characteristics, properties, and physiological actions. These amino acids are unique metabolically in that they are primarily catabolized by peripheral tissues (i.e. skeletal muscle) and have similar catabolic pathways (Voet and Voet, 1990). They are first transaminated to alpha-ketoacids by the enzyme, branched-chain alpha-ketoacid dehydrogenase. This step requires the aid of several coenzymes, thiamine pyrophosphate being the most prevalent. The end result is succinyl CoA for valine, acetyl CoA for leucine (the second of two exclusively ketogenic amino acids), and succinyl CoA or acetyl CoA for leucine, and succinyl CoA or acetyl CoA for isoleucine (Voet and Voet, 1990).

BCAA have a protein sparing effect when their levels are increased through infusion or oral administration. An overnight infusion of BCAA was associated with a 20–60% decline in arterial concentrations of the other amino acids. This suggested that the BCAA inhibit proteolysis in skeletal muscle and other body tissues (Ferrando et al., 1995; Louard et al., 1995). In addition to the protein sparing effects, leucine and the other BCAA exert their effects on respiratory functions. Investigations have shown that respiratory drive increased and diaphragm function improved when BCAA were administered (Manner et al., 1992). Increased levels of BCAA have been shown to decrease pCO2 and to stimulate the ventilatory response to hypercapnia. Also, a decrease in episodes of apnea in premature infants was found with an increase in BCAA (Blazer et al., 1994). Leucine metabolism has demonstrated an effect in the brain. Astrocytes in the brain metabolize leucine and its nitrogen furnishes the alpha amino group for glutamate synthesis. Glutamate sequesters free ammonia in the brain which is very sensitive to hyperammonemia. During this process, glutamate is converted to glutamine (Yudkoff et al., 1996; Yudkoff et al., 1994). This is an important step in the brain because glutamate functions as a neurotransmitter, regulates ammonia levels, serves as a constituent for glutathione and folic acid, and serves as a precursor of gamma-amino butyric acid (GABA) and other Krebs Cycle intermediates (Yudkoff et al., 1996; Yudkoff et al., 1994). However, glutamate is unable to cross the

blood brain barrier. Hence, leucine levels are significant in the overall nitrogen metabolism in the brain.

Methionine

Methionine undergoes an important metabolic reaction when it is converted to S-adenosylmethionine (SAM). SAM is the primary methyl group donor in one carbon metabolism (Voet and Voet, 1990). In SAM synthesis, L-methionine is coupled with ATP by the enzyme S-adenosylmethionine synthase with magnesium as a cofactor (Orten and Neuhaus, 1975). This produces the high energy SAM, which is unusual in that it contains no high energy phosphate bonds. All three high energy phosphate bonds in ATP are cleaved during the synthesis of SAM. The methyl group attached to the tertiary sulfur in SAM is irreversibly transferred to other molecules in a step catalyzed by methyltransferases.

Subsequent to transfer of the one carbon unit, SAM is hydrolyzed to homocysteine and adenosine by the addition of water to one of the sulfur bonds (Orten and Neuhaus, 1975). Homocysteine reacts with serine through cystathione synthase to form cystathione. Whereas cysteine and alpha-ketobutyrate are oxidated to form propionyl CoA and then subsequently succinyl CoA. The metabolism of valine, isoleucine, threonine, and methionine converge at succinyl CoA to enter the Krebs Cycle (Voet and Voet, 1990). Methionine is another example of an amino acid that is not essential as long as its essential precursor is present. That is, a decrease in methionine will cause a deficiency in cysteine (Newsholme and Leech, 1983). Homocysteine may also be used in the resynthesis of methionine, the initial precursor in the pathway. N5-methyl-THF donates a methyl group to homocysteine in a reaction requiring methylcobalamin. This results in the resynthesis of methionine and production of tetrahydrofolate. Methylcobalamin, the coenzyme, is a metabolite of vitamin B12 (Voet and Voet, 1990).

Methionine is also an important factor in the synthesis of choline (Zeisel and Blusztajn, 1994). Synthesis of choline requires the addition of three methyl groups from the activated form or methionine (SAM). Methionine deficiency causes an insufficient supply of choline even though choline can be resynthesized from phosphatidylserine in membranes (Zeisel and Blusztajn, 1994).

Lysine

The catabolism of lysine results in the formation of acetoacetate. It is unique in that neither of its amino groups undergo transamination as the first step in their catabolism. In the mammalian liver, lysine first forms alpha-aminoadipate-delta semialdehyde before it is converted to acetoacetate (Martin et al., 1983).

In rats, excess dietary lysine has a significant effect upon the distribution of carnitine and trimethyllysine (TML), a carnitine precursor. In a high lysine

diet, plasma concentration of carnitine is decreased while the concentrations of trimethyllysine are increased in the plasma and in skeletal muscle (Davis et al., 1993). TML has been shown to stimulate cell proliferation in bone marrow, intestinal tissues, and in cultured lymphocytes (Szende, 1993). L-lysine has also been shown to depress the central nervous system and to have anti-seizure properties. Similar to babiturates, lysine enhances [3H]flunitrazepam binding in the brain (Chang and Gao, 1995).

Threonine

When catabolized, threonine is dehydrated first to alpha-ketobutyrate, which is then converted to propionyl CoA, the precursor of succinyl CoA. Through threonine metabolism pyruvate is formed, which also enters the Krebs Cycle (Martin et al., 1983).

In diet-induced hyperthreoninemia, increased quantities of threonine and glycine were found in brain tissue. When moderate levels were administered, glycine did not increase in the brain. It was discovered that glycine was increased only at high levels of dietary threonine (Castagne et al., 1994). In the peripheral tissues, increased glycine concentrations again resulted from a high threonine diet. Hepatic threonine dehydrogenase activity was induced in these studies (Castagne et al., 1993). Rats fed a threonine imbalanced diet exhibited altered hepatic metabolism of long-chain free fatty acids (Fukuda et al., 1990). There was a 2–4 fold increase in triglyceride levels as well as enlarged livers. Excess threonine may be a causative factor in hypertension. Rats given and 8% threonine solution exhibited an elevated systolic blood pressure and a thickening of their small arterial walls (Vasdev et al., 1995).

Overview of the therapeutic effects of the essential amino acids

The therapeutic application of essential amino acids has received considerable attention in respiratory physiology, cardiology, renal failure, neurological disorders, and congenital defects. The benefits of exogenous essential amino acid therapy lies in the relative abundance and economy of these biologically active materials. This section deals with an overview of essential amino acid therapeutic applications.

Methionine

As discussed previously, methionine, a sulfur containing amino acid, is also very important in single-carbon metabolism in the activated S-adenosylmethionine (SAM) form. Methionine also plays a role in detoxification in the liver (Marchesini et al., 1992; Seyoum and Persaud, 1991). In chronic alcoholics, methionine metabolism is significantly altered (Seyoum and Persaud, 1991; Loguerico et al., 1994), producing a deficiency of SAM. This deficiency, observed in cirrhotic subjects, is located at the S-adenosylmethionine synthase step in methionine metabolism (Useman et al.,

1993). Investigators have considered the feasibility of giving exogenous doses of the methionine derivative, SAM, to ameliorate many of the complications observed in chronic liver diseases and cirrhosis (Seyoum and Persaud, 1991; Useman et al., 1993). Through therapeutic doses of SAM, glutathione would also be increased, leading to protection against oxidant stress from drug-induced hepatotoxicity (Useman et al., 1993; Ayala et al., 1991). Preliminary studies regarding this application have given promising results in both the improvement in liver function tests and biochemical parameters of choleostasis (Useman et al., 1993). Dietary supplementation of methionine also increases levels of other free-radical scavengers and lowers the succeptability to lipid peroxidation (Selvam and Kurien, 1992). Jaundiced patients showed a decrease in extrahepatic membrane cholesterol deposition due to exogenous SAM administration (Rafique et al., 1992). However, some liver pathologies, such as patients with intrahepatic choleostasis, have no response to SAM administration (Ribalta et al., 1991).

Amino acids have been known to enhance the pharmacological effects of some other drugs when given in together (Young, 1991). One such application of methionine has been observed in animal experiments involving lead intoxication in rats (Tandon et al., 1994). Ethylenediamine tetra acetic acid is a chelating agent used clinically in patients with heavy metal poisoning. Supplementation of this regimen with zinc and methionine increased urinary excretion of lead. However it did not reverse the lead-induced biochemical alterations (Tandon et al., 1994).

Therapeutically, methionine may be used clinically as a prophylactic to guard against congenital defects from teratogen exposure. Exogenous methionine has been shown to reverse the teratogenic effect of trans-retinoic acid in mice (Lau and Li, 1995).

Some cancer cells displayed a methionine-dependant growth pattern (Breillout et al., 1990; Pascale et al., 1995). The relationship between methionine dependency and the metastatic potential of a rat cancer line has been demonstrated experimentally. The greater the metastatic potential, the greater the concentration of methionine required to maintain growth (Breillout et al., 1990). That is, methionine dependent cancer cell lines lost their tumorigenicity when they were injected into rats fed a methionine-deprived diet. However, this special diet substituted homocysteine for methionine to maintain growth of normal cells. Exploiting this metabolic defect in the cancer cell lines may be of possible therapeutic value (Breillout et al., 1990). Similar anticarcinogenic effects were observed in other studies (Pascale et al., 1993). Also the re-establishment of physiological SAM levels in rats has been shown to inhibit protooncogene expression and prevent lesion development in vivo (Simile et al., 1994).

A derivative of methionine, methionine sulfoxamine, reduces cortical infarct size in rats after middle cerebral artery occlusion (Swason et al., 1990). The mean volume of the infarct in the cortex was reduced by 33% in the group treated with the sulfoxamine analogue. Sulfoxamine also enhances brain glycogen stores (Swason et al., 1990). This finding could provide a new therapeutic approach for stroke patients.

Tryptophan

Tryptophan is the precursor of 5-hydroxy-tryptophan (serotonin), a potent effector of mood and behavior as well as a neurotransmitter or neuromodulator (Mousseau, 1993). Peripheral tryptophan metabolism directly affects plasma tryptophan availability and consequently serotonin synthesis (Serres et al., 1995). Therefore, tryptophan levels have an effect on depression and mood. Serotonin, a monoamine, is related to the catecholamine hormones norepinephrine and epinephrine. The therapeutic applications of tryptophan and its tryptamine metabolite are dependent on their physiological actions of regulating mood, sleep, motor activity, thermoregulation, sexual activity, aggression, feeding, learning, and memory. Hence, tryptophan is a highly significant biological molecule (Halberg, 1969).

The determination of tryptophan and serotonin concentrations in plasma and CSF have been a valuable diagnostic aid in the study of drugs that alter serotonin metabolism (Eynard et al., 1993; Salomon et al, 1993). One of the main therapeutic applications for tryptophan is its enhancement of monoamine oxidase inhibitors in the treatment of depression (Young, 1991). Tryptophan levels are one of the most frequently used end-points in diagnostic and neuropsychopharmacological studies of serotonin function (Price et al., 1990). Tryptophan depletion, which reduces brain serotonin function, is known to reverse the pharmacological action of particular serotonin reuptake inhibitors (SRI), but not drugs that inhibit norepinephrine reuptake (Salomon et al., 1993; Moller et al., 1990). Examples of drugs in this SRI category which are altered by reduced tryptophan levels include paroxetine and clomipramine (Moller et al., 1990). Rapid depletion of tryptophan in the diets of untreated depressed patients did not result in an immediate change of mood, but did increase serotonin levels on the day after the depletion test (Delgado et al., 1994). Other investigators reported little change in depressed mood upon tryptophan depletion in drug-free depressed and healthy subjects (Salomon et al., 1993). This suggests that reduced serotonin levels does not linearly relate to depression, but may have a predisposing effect.

Pathological and immunological effects have been reported from exogenous L-tryptophan administration. Eosinophilia-myalgia syndrome (EMS), associated with the ingestion of exogenous tryptophan, is characterized by myalgia, eosinophilia, chronic cutaneous lesions, progressive neuropathy, and myopathy (Bartz-Bazzanella et al., 1992; Priori et al., 1994; Varga et al., 1993). Ingestion of exogenous L-tryptophan has also been found to induce pancreatic atrophy (Love et al., 1993). L-tryptophan, along with other amino acids, has been shown to induce cholecystokinin (CCK) production and subsequent pancreatic enzyme production in dogs and humans (Singer et al., 1976). Tryptophan is the most potent amino acid for stimulating pancreatic synthesis activity in dogs. Other studies have implicated tryptophan and its metabolites in fibrosing illnesses like carcinoid syndrome, eosinophilic fasciitis and scleroderma (Fries et al., 1973; Hankes et al., 1977; Stachow, 1978; Stachow et al., 1985).

Although tryptophan may have therapeutic applications in combination with antidepressants, other therapeutic uses should be considered with caution due to the possible deleterious effects observed from exogenous tryptophan ingestion.

Branched-chain amino acids

Valine, leucine, and isoleucine are similar in both their metabolism and therapeutic applications. Neurophysiological therapeutic effects have been found with branched-chain amino acid (BCAA) administration. Patients who suffered from chronic liver failure or portal circulation defects showed a variety of neurological symptoms due to an increase in nitrogenous ammonia in the systemic circulation. This conditions is commonly referred to as hepatic encephalopathy and is characterized by mood or personality changes, drowsiness, coma, dysphasia, and asterixis (Rubin and Farber, 1988). Clinical trials have explored the psychotropic effects of the BCAA and their antagonistic action in encephalopathy (Rubin and Farber, 1988). Neurophysiological improvements have been confirmed in quantitative psychometric tests (Higuchi et al., 1994; Plauth et al., 1993).

Dietary restriction of phenylalanine is essential in preventing brain damage in PKU patients (Berry et al., 1990). BCAA have been used to treat phenylketonuria by inhibiting the entry of phenylalanine into the brain (Berry et al., 1990). Thus, there is a reduction of CNS toxic effects caused by phenylalanine. The data from clinical experimentation has shown that BCAA are useful and effective in maintaining low serum phenylalanine levels (Berry et al., 1990). Since BCAA have an inhibitory effect on proteolysis, they have also been used as a treatment in septic pateints and those with catabolic disorders (Jimenz et al., 1991). Leucine has a metabolic application in the determination of protein turnover rate, where patients are primed with isotopes of leucine (Reinartz et al., 1995). A synthetic analog of leucine is successfully used in the clinical treatment of duodenal peptic ulcer hemorrhage (Georgadze et al., 1990). Patients in this study were administered a 3 mg dose daily. Only 8% of the subjects failed to respond to this treatment while 32% had a positive result.

Isoleucine has a very low toxicity at pharmatological levels up to 8% solution concentration in rats. Body weight, hematology, and food consumption by rats were not altered, although an increase in urine output and relative kidney size was observed (Kawabe et al., 1996). Conversely, when BCAA were eliminated from diets in the treatment of Maple Syrup Urine Disease, increased proteolysis was observed with isoleucine deficiency (Giacoia and Berry, 1993). This suggests that there is a risk when specific amino acids are eliminated from the diet without adequate supplementation with other intermediates.

Besides phenylalanine, BCAA have an inhibitory effect on the transport mechanism of other amino acids. Valine inhibits the transport of tryptophan across the blood-brain barrier (Williamson et al., 1995). Since tryptophan is the precursor of 5-hydroxytryptamine (serotonin), a mild decrease in brain

serotonin neurotransmission was observed with subsequent lowering of mood (Williamson et al., 1995).

Valine deficient diets were shown to increase calcium excretion in urine with a reduction in bone mass in chicks (Farran and Thomas, 1992). BCAA deficient diets have been studied for possible use in anti-tumor therapy (Nishihira et al., 1993). These investigators have demonstrated that valine and leucine deficient diets had the most desirable effect in decreasing tumor growth with minimal loss of body mass. However, diets deficient in all three BCAA had less effect on inhibiting tumor growth and negatively impacted host development. This suggests that selective removal of amino acids from the diet would be more beneficial in tumor therapy with less weight loss than elimination of all three BCAA. Vitamin B12 deficiency causes neurological deterioration if left untreated. The administration of valine and isoleucine, two precursors in the propionic acid pathway, protected against neurological damage in a study group of B12 deficient bats (Vieira-Makings et al., 1990). It appears in this study that valine and isoleucine circumvented this by stimulating the propionic acid pathway.

Arginine

The therapeutic applications of arginine are numerous systemically. As previously discussed, arginine is converted to nitric oxide (NO) which acts as a mediator in vasodilation (Bode-Boger et al., 1996), congestive heart failure (Koffman et al., 1995), inflammatory response (Kelley et al., 1995), chemotherapy (Birttenden et al., 1994), inflammatory pulmonary disease (Jorens et al., 1993), pulmonary hypertension (Castillo et al., 1995), and axon growth (Cestaro, 1994). Arginine reduces postischemic injury in the heart and exerts antihypertensive and antiproliferative effects on vascular smooth muscle (Nakaki and Kato, 1994). Arginine also prevents local vasospasms, unwanted proliferation of smooth muscle cells, and helps to control blood coagulation (Schini and Vanhoutte, 1993). The nitric oxide synthase pathway, in which arginine is a precursor, has been implicated in the pathogenesis of septic shock, hypertension, and atherosclerosis (Palmer, 1993). Studies in dogs have shown that a cardioplegic solution supplemented with L-arginine reduces the infarct size, preserves postischemic systolic and diastolic regional function, and prevents arterial endothelial dysfunction (Sato et al., 1995). Without L-arginine supplementation, ischemic damage and contractile dysfunction remained (Sato et al., 1995). This study suggests that L-arginine may be a possible treatment in heart attack patients. Capillary reperfusion with exogenous arginine after ischemic conditions in hamsters further illustrates its effect on vascular function (Bertuglia et al., 1995).

Arginine has been used in the treatment of necrotic colitis, which is caused by an enterotoxin whose symptoms exhibit an infarction of the mucosa, edema, and hemorrhage (Rubin and Farber, 1988). The neuroeffector action causes smooth muscle relaxation, while nitric oxide maintenance of the intestinal mucosa protects the gut from blood-born toxins and tissue destructive mediators. Thus, L-arginine may be considered as a potential therapy for

necrotic enterocolitis (DiLorenzo et al., 1995). Arginine supplementation has also been used in the prevention and treatment of osteoporosis. When pharmacological doses of arginine are administered, growth hormone, IGF-1, and NO responses are induced (Ghigo et la., 1994). Both GH and IGF-1 are important mediators of bone deposition (Visser and Hoekkman, 1994). Therefore, L-arginine administration may be part of an effective treatment to increase bone mass.

Arginine is also involved in the inflammation, tissue repair, and fibrogenesis processes in the kidney as described by (Ketteler et al., 1994). Arginine is essential for the synthesis of several metabolites that are secreted in the kidney such as creatine, urea, and nitric oxide which is excreted as nitrates and nitrites (Reyes et al., 1994). Dietary supplementation of arginine has resulted in improvements of several kidney pathologies. These pathologies include subtotal nephrectomy, diabetic nephropathy, cyclosporin A administration, salt-sensitive hypertension, uretal obstruction, puromycine amino-nucleoside nephrosis, kidney hypertrophy due to high-protein diets, and glomerular thrombosis (Reyes et al., 1994).

Lysine

Lysine has been used in the treatment of recurrent herpetic lesions (Wright, 1994). The herpes simplex virus requires high concentrations of arginine to synthesize proteins and replicate. Lysine acts as an anglogue of arginine and competes at the site of absorption in the small intestine. Consequently, lysine prevents the development of the herpes labialis lesions. Additionally, lysine is effective in the treatment of recurrent aphthous ulcers (Wright, 1994).

Lysine acetylsalicilate (lysine aspirin) and bendazac lysine (a lysine salt), both derivatives of lysine, have been investigated for their therapeutic effects on migraine headaches (Chabriat et al., 1994), acute respiratory infections (Barberi et al., 1993), and rheumatoid arthritis (Hills et al., 1990). Given in combination with metoclopramide, lysine aspirin proved to be an effective treatment for migraine headaches (Chabriat et al., 1994). In the treatment of acute respiratory infections (laryngitis, tracheitis, bronchitis, pneumonia) the effects of lysine aspirin was comparable to that of nimesulide (Barberi et al., 1993). However, patients did incur more gastrointestinal side effects (Barberi et al., 1993). When comparing lysine aspirin with ibuprofen for the relief of rheumatoid arthritic symptoms, more side effects were found with the lysine aspirin (Hill et al., 1990). However, the majority of patients preferred the lysine aspirin therapy over ibuprofen since there was improved mobility and the alleviation of pain (Hill et al., 1990). The bendazac derivative of lysine may be useful in delaying the progression of cataract formation since it inhibites the denaturation of proteins (Balfour and Clissold, 1990).

It has been reported that L-lysine supplementation can significantly enhance intestinal calcium absorption and improve renal resorption of filtered calcium (Civitelli et al., 1992; Furst, 1993). Since age-related bone loss is due to calcium deficiency, L-lysine may prove to be an effective treatment modality.

Histidine

As discussed previously, histidine is a precursor of histamine, the mediator of many inflammatory and allergic responses (Newsholme and Leech, 1983). Histidine also has oxygen free radical scavenging qualities. An investigation of the antioxidative properties of histidine in myocardial injury in rat hearts showed that histidine prevented postischemic reperfusion injury (Chabriat et al., 1994). Hearts treated with histidine showed greater functional recovery with increased high-energy phosphates. Histidine was shown to quench hydroxyl radicals and hydrogen peroxide, but not superoxide anions (Cai et al., 1995). Another study involving subarachnoid hemorrhage in rabbits showed that histidine attenuated cerebral vasospasm (Fadel et al., 1995). In a study on the effects of histidine on brain edema and cardiac function after thrombotic ischemia in rats, it was found that diminished histidine decreased brain water content and enhanced left ventricular function in animals (Li et al., 1995).

Histidine-containing dipeptides, carnosine and anserine, had an anti-inflammatory effects and may be valuable in the wound healing process (Bolldyrev and Severin, 1990). It is believed that these dipeptides interact with oxygen radicals and lipid peroxidation products to prevent membrane damage.

Phenylalanine

Phenylalanine and its metabolite tyrosine are involved in the initial metabolic steps in dopamine synthesis. An abundance of research has focused on the modulation of phenylalanine in patients with phenylalanine hydroxylase deficiency (PKU) (Pietz et al., 1995; Lou, 1994; Leuzzi et al., 1995). As a result of this genetic aberration, clinical literature concerning phenylalanine is involved in the manipulation and metabolism of PKU patients (Wang, 1991). Analysis of nucleic material coding for the phenylalanine hydroxylase gene has been of some benefit in predicting the outcome and severity of phenylketonuria (PKU) in humans (Guttler and Guldberg, 1994; Guldberg et al., 1994). Some success has been achieved in correcting the phenylalanine hydroxylase genetic deficiency through transduction of mice hepatocytes in vitro to produce dramatically higher levels of phenylalanine hydroxylase (Lui et al., 1992). Another genetic aberration in phenylalanine metabolism has been elucidated in its predisposition for hypertension and stroke, which might be related to excessive stimulation of the sympathetic nervous system (Zhao, 1991).

A derivative of phenylalanine, 4-borono-2-[18F] fluoro-D, L-phenylalanine has shown promise as a tracer in positron emission tomography (PET) for imaging of cancer cells (Ishiwata et al., 1991; Ishiwata et al., 1992). Phenylalanine has also been used diagnostically to determine protein synthesis in visceral tissue (Southhorn, et al., 1992). This method of measuring protein synthesis represents an improvement over continuous infusion methods in visceral tissues.

Cirrhosis of the liver is associated with elevated phenylalanine levels due to increased proteolysis and decreased splanchnic extraction of dietary phenylalanine (Tessari et al., 1994). In Cirrhosis, the ratio of branched-chain amino acids to phenylalanine is altered although the mechanism for this is not completely understood (Tessari et al., 1993). The phenylalanine metabolites, phenylethylamine and phenylacetic acid, are involved in encephalopathy in sepsis and hepatic failure (Mizock et al., 1990).

Threonine

Therapeutically, threonine has been used as a treatment for spinal spasticity and multiple sclerosis (Hauser et al., 1992; Lee and Patterson, 1993). Threonine enhances the glycinergic postsynaptic inhibition of the motor reflex arc in the spinal cord with no toxic or adverse effects (Testa et al., 1992). When compared with commonly used antispastic drugs, which commonly cause sedation and increased motor wakness, threonine appears to be an appealing alternative (Hauser et al., 1992). In Amyotrophic Lateral Sclerosis, a fatal neurological disease with no known cure (Roufs, 1991), patients administered threonine complained less frequently about disease-related respiratory failure (Testa et al., 1992).

Although threonine consumption at levels of four times that normally found in the diet of rats did not impair their behavioral development (Castagne et al., 1995), threonine metabolism may have a role in mediating hypertension. Specifically, ethanol and threonine are precursors of acetaldehyde. Upon long-term dietary supplementation of threonine in rats, smooth muscle cell hyperplasia increased while luminal diameters of small arteries and arterioles decreased (Vasdev et al., 1995). Thus it is apparent that acetaldehyde may be is implicated as the mediating factor in both ethanol and threonine-induced hypertension.

Conclusion

The ten essential amino acids are responsible for a vast array of metabolic, physiologic, and therapeutic effects throughout the body. In addition to their roles in peptide and protein structure, these free amino acids have significant functions as specialized nitrogen containing products, neurotransmitters, and as alternate energy sources via the Krebs Cycle. Unique therapeutic uses of these biologically significant molecules are currently being explored and could become an economic alternative to more expensive clinical approaches. Since these biologically active amino acids must be obtained from the diet, an over abundance or deficiency in just one of these may have severe pathological consequences. Even though the metabolic, physiologic, and therapeutic effects of these essential amino acids have been extensively explored in some areas, they still remain an important modality in clinical medicine. The negative systemic effects observed with pharmacological doses of some amino acids, such as tryptophan, may limit their use clinically. However, the diverse

physiological and metabolic applications of many of the essential amino acids will certainly yield a significant body of cost-effective alternative therapeutic applications.

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Received May 6, 1997