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SYMPOSIUM ON 'AMINO ACID METABOLISM IN CLINICAL MEDICINE'

Some recent developments in the study of amino acid metabolism

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The amino acids of dietary proteins are precursors for the synthesis of tissue proteins and many nitrogen-containing, sulphur-containing and aromatic compounds with unique biological activities. Amino acids not used for these purposes are degraded rapidly; their N, which is released as ammonia or is transferred to keto acids to form alanine, aspartate or glutamate, is ultimately incorporated into urea; the carbon skeletons are oxidized directly for energy or are converted to glucose or fatty acids. With this wide variety of metabolic reactions to choose from, a brief discussion of 'developments in the study of amino acid metabolism' must be highly selective and will undoubtedly reflect the interests of the author. I have elected to discuss some aspects of: control of tissue protein synthesis and degradation by amino acid supply; control of the degradation of phenylalanine, branched-chain amino acids (BCAA) and methionine; the use of amino acids as energy sources by the intestine; and, the role of amino acids in the control of feeding behaviour.

Control in the metabolism of amino acids

Few unique regulatory systems for control of enzymes of amino acid metabolism have been identified. Metabolic control is considered to depend mainly on substrate concentration in relation to the Michaelis-Menten constants (K_m) of the enzymes for the initial steps of protein synthesis and amino acid degradation, and on the tissue concentrations of the catabolic enzymes.

It has long been recognized, from observations that the N of appropriately-balanced dietary proteins is used very efficiently for growth by mammals when protein intake is below the requirement (Block & Mitchell,

1946–47), that amino acids must be channelled preferentially into pathways for the synthesis of tissue proteins. Only when the quantities of amino acids consumed exceed the requirements for protein synthesis are appreciable amounts of amino acids oxidized for energy (Kang-Lee & Harper, 1978; Patterson & Harper, 1982). Among the factors that contribute to the efficiency of this channelling are: (1) the enzymes for degradation of essential amino acids, other than the BCAA, are confined almost exclusively to the liver (Miller, 1962); (2) the K_ms of the aminoacyl-tRNA synthetases are much lower than those of the amino-acid-degrading enzymes so, when tissue amino acid concentrations are low, the former should be saturated with substrate and be fully active, whereas the rates of reaction of the latter will be low (Harper, 1971, 1974; Krebs, 1972; Rogers, 1976); (3) insulin released after ingestion of a meal stimulates uptake of amino acids into cells of tissues such as muscle in which few essential amino acids are degraded and also stimulates protein synthesis (Harper, 1974). Yet, Jefferson & Korner (1969) observed maximum rates of protein synthesis in an in vitro system from perfused liver only when amino acid concentrations in the perfusate were increased several-fold above post-prandial arterial concentrations. A logical inference from these observations was that amino acids exerted control over the rate of protein synthesis in ways other than by serving as substrates for aminoacyl-tRNA synthetases.

Amino acids in the control of protein synthesis and degradation

Current knowledge of nutritional control of protein synthesis has been summarized by Henshaw (1980) and Austin & Clemens (1981). In starved animals and in animals deprived of protein or a single amino acid, the rate of protein synthesis in several tissues, but especially in muscle, falls. This is associated with disaggregation of polysomes and an accumulation of ribosomal subunits. In mammalian cells in tissue culture, these responses to amino acid deprivation are rapid and recovery occurs equally rapidly when the missing amino acid is provided. Recovery occurs even if new RNA synthesis is inhibited by actinomycin D, indicating that control by amino acid supply is exerted at the level of translation of mRNA rather than at the level of transcription.

The presence of uncharged tRNA or depletion of ATP has been shown in cultured cells to depress protein synthesis. Measurements made in starved animals, nevertheless, indicate that starvation will depress protein synthesis even when tRNA is almost all acylated; depression of protein synthesis is also observed under conditions in which ATP is not depleted. Pain & Henshaw (1975) and Pain *et al.* (1980) observed that, in Ehrlich cells deprived of lysine, the number of initiation complexes (40S Met $tRNA_f$) was substantially reduced, and that protein synthesis was stimulated by addition of initiation factor eIF-2. Taken altogether these observations suggest that the block in protein synthesis in response to deprivation of one or more amino acids occurs in reactions involved in the attachment of ribosomes to mRNA and is the result of a reduced rate of initiation.

Flaim, Liao *et al.* (1982) and Flaim, Peavy *et al.* (1982) have recently

reinvestigated the effects of amino acid supply on the rate of protein synthesis in the isolated perfused liver. They reproduced their earlier results (Fig. 1) showing that, for the maximum rate of protein synthesis, the liver required five times the normal plasma amino acid concentrations. They found that tRNA was fully charged even when protein synthesis rate was low and that mRNA was not depleted and was functional. A high proportion of polysomes, however, were disaggregated when amino acid concentrations in the perfusate were low. They concluded that initiation of protein synthesis was suppressed. When they examined the effect of perfusate amino acid concentrations on the ability of the 40S ribosomal subunits to bind [^{35}S]methionine, a measure of the formation of 40S initiation complexes, they found twice as much radioactivity per unit of RNA in the 40S fraction from livers perfused with amino acids as in that from livers perfused without amino acids.

The mechanism by which amino acid supply affects initiation complex formation is not known. Clemens (1983) has speculated, on the basis of recent observations by Damuni *et al.* (1982) showing that the activities of aminoacyl-tRNA synthetases are controlled by a phosphorylation–dephosphorylation process and that activation of this process occurs in response to feeding, that there may be a link between activation of these enzymes and control of the initiation process.

Regulation of tissue protein degradation by amino acid supply would appear to be as important as regulation of protein synthesis in the control of tissue protein content. It deserves equal attention. Recent observations by Poso *et al.* (1982)

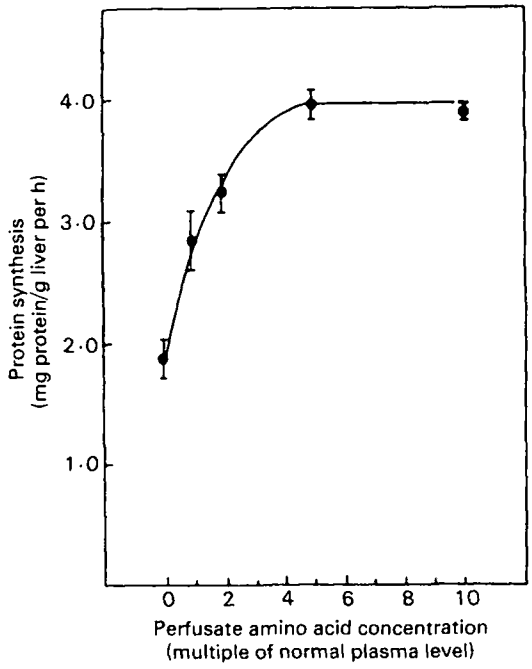

Fig. 1. Rates of protein synthesis in perfused liver in response to variations in amino acid concentration in the perfusate (Flaim, Peavy *et al.* 1982).

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indicate that eight amino acids (leucine, phenylalanine, tyrosine, glutamic acid, proline, histidine, tryptophan and methionine) will suppress intracellular proteolysis in the isolated perfused liver. The amino acids differ greatly in effectiveness. Leucine is most effective; its keto acid, α-ketoisocaproic acid, is also highly effective.

These observations on the effects of amino acid supply on protein synthesis and degradation illustrate that amino acids serve as regulatory components of important metabolic control systems. Unravelling the mechanisms by which amino acid supply controls protein synthesis and degradation has proved to be a complex and difficult problem, but progress is being made towards providing a description of the mechanisms by which homoeostosis of tissue protein content is maintained.

Control of amino acid catabolism

The rate of catabolism of amino acids increases directly with increasing amino acid intake after intake exceeds the requirement for protein synthesis but, at some point, the capacity of the degrading systems is exceeded and food intake is depressed (Anderson *et al.* 1968). Degrading-enzymes for most amino acids are induced when protein intake reaches this point; then, as the capacity for amino acid catabolism increases, food, and hence protein, intake increase but plasma and tissue amino acid concentrations, except for those of BCAA, are maintained at or below normal levels despite greatly excessive protein intake. Changes in the rate of amino acid catabolism when protein intake is altered can thus be explained, for the most part at least, as the result of responses to changes in the concentration of substrate and in enzyme content. However, the limiting enzymes for catabolism of phenylalanine, the BCAA (leucine, isoleucine and valine) and methionine, which are depleted when rats are given protein-deficient diets, are not induced to high levels when rats consume high-protein diets; yet, phenylalanine and methionine do not accumulate in the body fluids of rats that have adjusted to a high-protein intake. Also, although the concentrations of BCAA do rise as protein intake of the rat increases, the increases in the free pools of BCAA in the body represent only a small fraction of the additional intake.

Observations made during the past few years indicate that the activities of phenylalanine hydroxylase (*EC* 1.14.16.1) and branched-chain keto acid (BCKA) dehydrogenase (*EC* 1.2.4.4), unlike those of other amino-acid-degrading enzymes (at least insofar as we know at present), are controlled by unique regulatory systems, and for methionine there are at least two degradative pathways.

Phenylalanine hydroxylase. Phenylalanine hydroxylase catalyzes the tetrahydropteridine-dependent hydroxylation of phenylalanine to tyrosine. Several investigators have reported that this enzyme, which is ordinarily rate-limiting for phenylalanine catabolism (Milstien & Kaufman, 1975), is not fully active in vivo (Donlon & Kaufman, 1980; Shiman & Gray, 1980; Shiman & Jefferson, 1982). The enzyme is not induced to high levels in animals given a high-protein diet but can be activated in vitro by a phosphorylation–dephosphorylation mechanism (Donlon & Kaufman, 1980) and in a variety of other ways in vitro (Hasegawa & Kaufman,

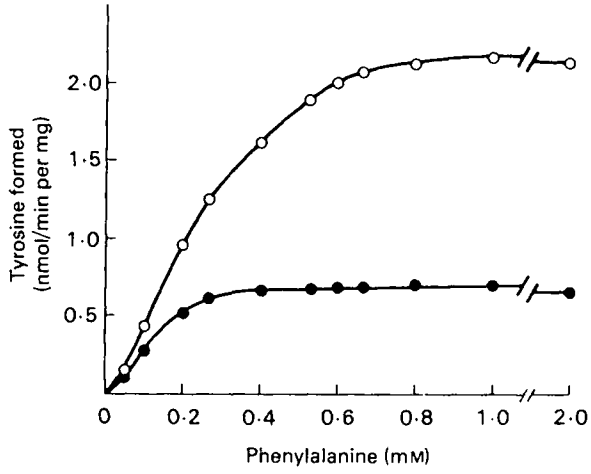

Fig. 2. Effect of substrate concentration on phenylalanine hydroxylase (*EC* 1.14.16.1) activity in crude liver extract before and after activation (Hasegawa & Kaufman, 1982). ●, Before activation; ○, after activation (incubation at 25° for 60 min).

1982); it can also be activated by its substrate (Shiman & Gray, 1980); by iron (Shiman & Jefferson, 1982); and by a Mn^{2+}-sensitive system. The type of response observed is shown in Fig. 2.

The physiological significance of the various activation systems has not been established but from observations on the activation of phenylalanine hydroxylase by glucagon treatment of rats, the *c*-AMP-dependent phosphorylation–dephosphorylation mechanism at least is evidently involved in the regulation of phenylalanine catabolism in vivo (Hasegawa & Kaufman, 1982). The hydroxylation system is activated, according to Abita *et al.* (1980), by glucagon concentrations in the physiological range. Also, glucagon treatment of rats stimulates phenylalanine metabolism in vivo (Haley & Harper, 1982).

The allosteric activation of purified phenylalanine hydroxylase by concentrations of phenylalanine below 0·1 mM (Shiman, 1980) would suggest that this mechanism should be functional in vivo also. The rate of catabolism of phenylalanine should also increase in response to increasing substrate concentration up to 1·0–2·0 mM-phenylalanine, as the K_m of the enzyme for phenylalanine is of the order of 0·2–0·3 mM. Hasegawa & Kaufman (1982) have reported that the combined effects of phosphorylation and the Mn^{2+}-sensitive activation process can result in an almost twenty-fold increase in the activity of the hydroxylase in crude enzyme preparations. As the rate of clearance of a large load of phenylalanine by both rats and human subjects is rapid, this would be explained most readily by activation of the enzyme by a number of these systems in vivo.

BCKA dehydrogenase. The first two steps in the degradation of the BCAA are transamination, which is reversible, followed by oxidative decarboxylation of the BCKA produced, which is irreversible. Enzymes for BCAA degradation occur in all organs and tissues studied. Responses of both the BCAA aminotransferase (*EC*

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2.6.1.42) and the BCKA dehydrogenase to dietary and hormonal treatments, except for declining activity associated with protein deficiency, have proven to be relatively small and not consistent. Oxidative decarboxylation of the BCKA, the first irreversible step in the degradative pathway, is considered to be the reaction at which BCAA catabolism is controlled (Harper & Zapalowski, 1981).

During the past few years it has become evident that the enzyme catalyzing this reaction, BCKA dehydrogenase, is subject to regulation by a phosphorylation–dephosphorylation mechanism (see Fatania *et al.* (1982) for references) and that the enzyme is not ordinarily fully activated in vivo (Gillim *et al.* 1983). Evidence for this has come from studies in which enzyme activity has been measured under various conditions: with and without preincubation of tissue preparations; with and without the inclusion of sodium fluoride, a phosphatase inhibitor, during preparation of the tissue enzyme source; with and without alkaline phosphatase (*EC* 3.1.3.1) added to tissue extracts prepared in a way that removes the phosphatase. The values in Table 1 from the study of Gillim *et al.* (1983) illustrate the effects of protein intake on the activity of BCKA dehydrogenase in three different tissue preparations assayed in the presence and absence of alkaline phosphatase. These responses to a high-protein intake should increase the capacity of rats fed on a high-protein diet to degrade BCAA.

BCKA dehydrogenase in the livers of rats fed on a low-protein diet is also activated within a short time after the animals have consumed a high-leucine diet (Table 2). These observations were made as part of an investigation of the basis for the depletion of isoleucine and valine and their respective keto acid pools in rats fed on high-leucine diets. Activation of the dehydrogenase by leucine could account for the increased rate of valine oxidation observed when rats were fed on a high-leucine, low-protein diet and this in turn could account for the depletion of tissue pools of isoleucine and valine in such animals (R. J. Block and A. E. Harper, unpublished observations).

Table 1. *Effect of protein intake on the extent of activation of branched-chain keto acid (BCKA) dehydrogenase* (EC 1.2.4.4) *in three tissues of the rat (after Gillim* et al. 1983)

		BCKA dehydrogenase activity (nmol NADH/min per g wet wt)		
Tissue	Protein intake (g/kg diet)	− Alkaline phosphatase	+ Alkaline phosphatase (*EC* 3.1.3.1)	% in active form
Heart	Low, 80	111	244	44
	High, 270	124	286	44
Liver	Low, 80	65	189	33
	High, 270	643	662	99
Kidney	Low, 80	200	385	54
	High, 270	332	471	70

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Table 2. *Effect of ingestion of a high-leucine meal on hepatic branched-chain keto acid (BCKA) dehydrogenase* (EC 1.2.4.4) *activity in rats consuming a low-protein diet (after R. J. Block and A. E. Harper, unpublished results)*

Treatment	BCKA dehydrogenase activity (nmol/min per g liver)		% in active form
	No preincubation	After preincubation	
Control (90 g casein/kg diet)	431	1002	43
High-leucine diet (control + 30 g leucine/kg diet)	1066	1332	80

Pathways of methionine catabolism. Methionine is known to be metabolized via the transsulphuration pathway (see Fig. 3), the initial reaction of which involves the formation of S-adenosylmethionine (SAM), the major methylating agent of the body. The subsequent formation of homocysteine occurs only after SAM has been used for transmethylation. The succeeding reactions, formation of cystathionine and its cleavage to yield cysteine, release the carbons of methionine into oxidative pathways. The further metabolism of cysteine yields a number of sulphur-containing compounds of biological importance (Mudd & Levy, 1978). This pathway has many of the characteristics of an anabolic pathway; it has a high energy requirement and is essential for the synthesis of many biologically important compounds. Also, the initial and rate-limiting enzyme has limited capacity and, unlike those of most amino-acid-degrading pathways, is not induced appreciably in animals fed on a high-protein diet. These thoughts and an interest in the high toxicity of methionine when it is fed in excess to animals led Benevenga and associates to examine the metabolism of this amino acid (Benevenga & Harper, 1970; Aguilar *et al.* 1974).

Observations that: (1) the rate of metabolism of the methionine methyl group to CO_2 was high and increased with increasing methionine intake; (2) dietary additions of choline and betaine, which should have diluted radioactivity from the methyl group of methyl-[^{14}C]methionine entering the transsulphuration pathway, failed to do so (Case *et al.* 1976); and (3) results of similar experiments with dietary addition of sarcosine indicated that less than 20% of the methionine methyl was oxidized via pathways that were dependent on sarcosine formation (Mitchell & Benevenga, 1976); all led to the conclusion that another pathway for methionine degradation that did not involve transsulphuration must exist.

Methionine has long been known to undergo transamination to α-ketomethiolbutyrate (KMB) and production of CO_2 from methyl-[^{14}C]methionine was suppressed by inclusion of the transaminase inhibitor, aminooxyacetate, in a rat liver homogenate system (Mitchell & Benevenga, 1978). Also, decarboxylation of KMB by the in vitro liver system was suppressed by inclusion of various α-keto acids, especially BCKA, that are decarboxylated by known liver decarboxylation systems (Dixon & Benevenga, 1980; Livesey & Lund, 1980). In other studies

(Steele & Benevenga, 1978), addition of unlabelled 3-methylthiopropionate suppressed the conversion of ^{14}C from methyl-[^{14}C]methionine to $^{14}CO_2$; also ^{14}C and ^{35}S of appropriately labelled methionine were recovered in methylthiopropionate. When the gas evolved from liver homogenates incubated with either methyl- or sulphur-labelled 3-methylthiopropionate was analysed, it was found to contain methanethiol and hydrogen sulphide (Steele & Benevenga, 1979). Accumulation of these products could account for the toxicity of high intakes of methionine. The pathway for methionine degradation based on these observations is shown together with the transsulphuration pathway in Fig. 3.

Studies with liver homogenates of the yields of CO_2 from the methyl carbon of methionine or SAM, indicated that five to eight times as much methionine is metabolized via the transamination as by the transsulphuration pathway. The proportion was higher when the methionine concentration in the medium was increased from 0·1 to 1·0 mM. Thus, with two pathways for catabolism of methionine, the capacity of the organism to degrade excess methionine is much greater than would be assumed if this amino acid were degraded only via the transsulphuration pathway. Details of the evidence for the transamination pathway have been summarized by Benevenga (1983).

Recapitulation. With evidence for: (1) unique control systems that can increase the proportion of phenylalanine hydroxylase and BCKA dehydrogenase existing in an active form; (2) two pathways for degradation of methionine; and (3) induction by a high-protein diet of the key enzymes for degradation of tryptophan, threonine, serine, histidine, lysine, arginine, tyrosine, alanine and glutamic acid, we are much closer than we were to being able to provide a comprehensive explanation of the ability of the rat, after a period of adaptation, to degrade rapidly the large quantities of amino acids consumed when it is fed on a high-protein diet.

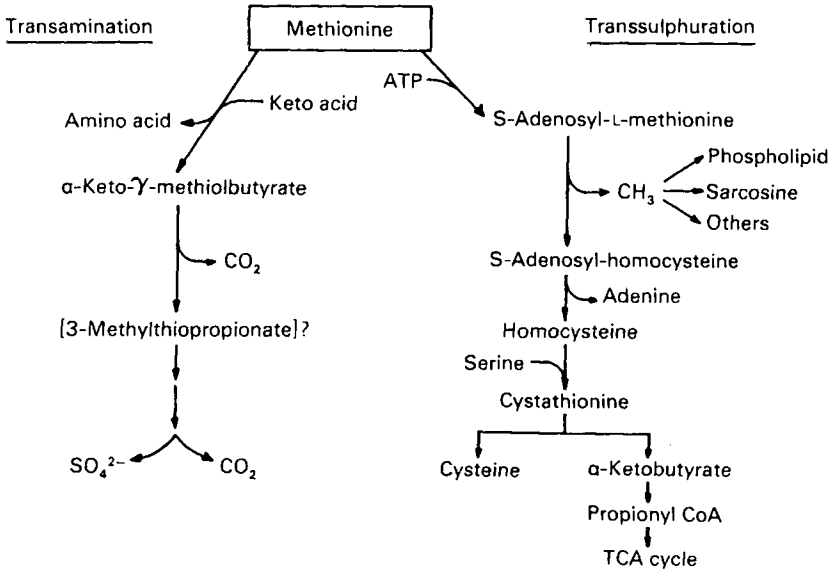

Fig. 3. Pathways of methionine metabolism.

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Amino acids as energy sources

Amino acids consumed in excess of the amounts needed for protein synthesis are oxidized preferentially in the liver as sources of energy (Krebs, 1972); during starvation, amino acids released from tissue protein degradation, especially by muscle, serve as substrates for glucose production (Cahill, 1970). Studies of amino acid oxidation in vivo in the rat indicate that amino acids are oxidized in proportion to intake even when most of the energy in the diet is provided from glucose (Kang-Lee & Harper, 1978). With recognition that BCAA are oxidized by muscle, the question arose as to the significance of BCAA as an energy source for this tissue. Calculations based on rates of oxidation of BCAA by the isolated perfused hind-quarter indicate that, in the rat, oxidation of these amino acids would account for only 2–5% of resting oxygen consumption (Hutson *et al.* 1980). Calculations based on the amount of BCAA released through protein degradation during starvation indicate that less than 2% of the fasting energy needs could be met from this source.

Dispensable amino acids make up more than half of the amino acids of both dietary and tissue proteins and several of these, glutamine, glutamate, aspartate and alanine, can be degraded by most tissues. Both alanine and glutamine serve as carriers for the removal of N released from muscle, and probably other organs and tissues, during degradation of amino acids, particularly the BCAA. Emphasis has usually been placed on the roles of these amino acids in transporting N to the liver for disposal as urea.

During the past few years Windmueller & Spaeth (1974, 1975, 1978, 1980) have been studying the nature of respiratory fuels used by the intestine. In 1974 they reported that intestinal glutamine concentration was low (200 nmol/g) compared with that of muscle (3300 nmol/g) and other tissues. They also observed that in the isolated, vascularly perfused intestine, glutamine was extracted from the blood in the presence of glucose and much of the C was converted to CO_2. They also noted that only a small fraction of glutamate and glutamine administered into jejunal segments *in situ* appeared unchanged in the venous blood leaving the intestine. As much as 60% of the C was recovered in the blood as CO_2. In their more recent studies (Windmueller & Spaeth, 1978, 1980) on intestinal segments with an intact arterial supply *in situ*, they have been able to quantify more completely, by measuring products released into the venous drainage, metabolites from the lumen and the blood used as energy sources by this tissue.

The contributions of arterial metabolites to CO_2 produced by the intestine perfused without nutrients in the lumen is summarized in Table 3. Windmueller & Spaeth (1978) estimated that 50% of the arterial glutamine was converted to CO_2 by the intestine. In subsequent studies with perfusion of nutrients into the lumen they found that 39% of the CO_2 formed was from lumenal aspartate, glutamate and glutamine. They calculated that the combined contributions of these amino acids from the blood and the lumen represented 77% of the CO_2 produced.

These observations provide evidence of a unique role for several dispensable

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Table 3. *Contribution of arterial metabolites to carbon dioxide released from jejunal segments with intact arterial blood supply (after Windmueller & Spaeth, 1978, 1980)*

Metabolite	CO_2 yield (nmol/min per g)	% of total
glutamine	597	35
3-hydroxybutyrate	444	26
acetoacetate	410	24
glucose	116	7
lactate	79	5
unesterified fatty acids	58	3

amino acids as energy sources for the intestine. As this group of amino acids represents the major portion of the free amino acid pools of most tissues, the possibility that they serve other unique functions deserves greater attention.

Amino acids and feeding behaviour

Changes in feeding behaviour (food intake and preference) are not usually considered to be responses controlled by the system for regulation of amino acid metabolism; yet, depressed food intake and altered food preferences are early responses of animals to marked changes in either the protein content or proportions of amino acids in the diet (Harper *et al.* 1970). Changes in feeding behaviour as the result of altered dietary amino acid patterns are associated with depletion of the brain pool of an amino acid owing to competition among plasma amino acids for uptake into the brain (Peng *et al.* 1972). Also, changes in the protein content of the diet result in alterations in brain trytophan uptake and brain content of the neurotransmitter serotonin as the result of competition between tryptophan and other large neutral amino acids during transport to the brain (Fernstrom & Wurtman, 1972). Observations of this type have created interest in associations among diet, blood and brain amino acids, brain neurotransmitters, and amino acid metabolism in relation to feeding and other behaviour.

Evidence (Fernstrom & Wurtman, 1972) that brain serotonin content was directly proportional to the ratio of the plasma concentrations of tryptophan to that of other large neutral branched-chain and aromatic amino acids (trp:LN), and observations (Ashley & Anderson, 1975) that long-term protein intake was inversely proportional to plasma trp:LN, led Anderson (1979) to propose that changes in protein intake of rats selecting between two diets that differed in protein content were mediated by changes in brain serotonin content. In a study designed to test this hypothesis, Peters & Harper (1981) found no significant correlation between brain serotonin content and the amount of protein selected by rats during a 13-d period when they were allowed to choose between two diets differing in protein content.

In subseqent studies (Peters, 1983) in which rats were fed on single diets ranging widely in protein content, even though food intake was depressed when the casein

content of the diets exceeded 35%, no significant correlation between protein intake and brain serotonin content was observed in either short-term or long-term studies. In the longer term study, consumption of the diets with higher protein content increased after a few days. This was associated with increased activities of some amino-acid-degrading enzymes and reduced plasma and brain concentrations of essential amino acids other than BCAA, which increased. As a result, brain total essential amino acid concentrations were maintained within a narrow range despite a six-fold range in the protein intakes of groups fed on the different diets. In the long-term study, although no correlation between protein intake and whole brain serotonin content was observed, an inverse correlation between protein intake and plasma trp:LN was evident, largely as the result of incremental increases in plasma BCAA concentrations with increasing protein intake. The inverse correlation observed between protein intake and plasma trp:LN of rats that have become adapted to different dietary levels of protein, is evidently a reflection of the different amounts of protein they have consumed, not a determinant of subsequent protein intake. Li & Anderson (1982) have recently come to a similar conclusion from studies of the effects of diet on diurnal changes in brain tryptophan and serotonin concentrations.

It appears, from the results of several of these studies that, when the protein content of the diet approaches a level that will overload amino-acid-degrading systems, the protein intake of the rat is adjusted downward to a level that will prevent an undue rise in brain amino acid concentrations. This occurs through reduced food intake if only a single diet is offered or through selection of a lower-protein diet if a choice is available. As rats adapt to a high-protein diet, their amino-acid-degrading capacity increases and, although protein intake may increase greatly, neither plasma nor brain amino acid concentrations are further elevated. Adjustments of protein intake or preference under these conditions are apparently responses to a feedback system that controls ingestion of protein to an amount that will not overload either the amino acid catabolic system or the brain. The signal that mediates these adjustments in protein intake and preference has not been identified.

Investigations of relationships among altered blood and brain amino acid concentrations, feeding behaviour and changes in brain neurotransmitter concentrations have potential for contributing to knowledge of how dietary, hormonal or drug treatment may modify brain neurotransmitter concentrations and of providing the basis for new therapeutic approaches to certain neurological and behavioural disorders (Wurtman *et al.* 1981).

Conclusion

The developments selected for discussion, only a few of many that might have been selected, represent examples of research that is revealing the extent of integration that exists in the control of amino acid metabolism in vivo; how amino acid supply can control the rates of both anabolic and catabolic reactions; and how the metabolic state of the organism, by influencing protein consumption, can, in turn, control the quantity of amino acids that enters the metabolic system.

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