Animal Models of Amino Acid Metabolism: A Focus on the Intestine

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ABSTRACT

One important advantage of animal models is that they permit invasive approaches and can be especially valuable when evaluating tissue and specific features of metabolism in situ. The focus of this presentation is current models, which are providing insights into the pivotal importance of the gastrointestinal tract in amino acid metabolism. Intestinal amino acid metabolism is conceptually and technically difficult to approach and multiple processes must be accounted for: protein synthesis and degradation; transit of amino acids in both directions across the basolateral surface of enterocytes, in addition to uptake on the apical side; arterio-portal differences as well as net portal appearance during uptake of defined amino acid mixtures appearing on the luminal side; first pass amino acid metabolism. These key features are largely impossible to study without access to invasive approaches in vivo and cannot be reproduced in vitro. Douglas Burrin, Ron Ball, and Vickie Baracos and their co-workers have used the domestic piglet to study intestinal protein metabolism in situ in three distinctly different and complementary approaches. Collectively, their approaches allow a means to describe the key elements of intestinal amino acid capture (and release) and the means to probe their physiological and pathological variation. It seems evident that the portal-drained viscera represent sites of quantitatively important amino acid catabolism, and that this capacity combined with hepatic metabolism would largely limit the possibility of toxic sequelae of amino acids taken orally. J. Nutr. 134: 1656S–1659S, 2004.

KEY WORDS: • intestine • protein metabolism • amino acid metabolism • piglet • animal models

A critical barrier function

There is an impressive capacity for uptake of amino acids and free peptides from the lumen of the small intestine, and this capacity is so great that under most physiological and pathological circumstances it is not limiting. Enterocytes constitute “floodgates” of amino acid entry. However, the amino acid mixture available from the diet does not necessarily have any correspondence to requirements for these compounds. Because indispensable amino acids, particularly branched-chain, aromatic, and sulfur amino acids are toxic, absorbed amino acids cannot simply be released into the bloodstream. Tissues of the intestine capture, transform, and degrade amino acids and thus form a critical first line of defense against the vagaries of the dietary amino acid supply, acting in the capacity of “gatekeeper” as well. The gastrointestinal absorptive epithelium, followed in sequence by the liver, shares the responsibility of discharging an amino acid mixture into peripheral blood that is both qualitatively and quantitatively appropriate to support protein synthesis. In part, intestinal amino acid metabolism may simply reflect a large and metabolically active organ system, meeting its own requirements, as it performs the task of amino acid uptake on behalf of the organism. Because the overall nutrient requirements of this organ system are large, it makes sense that the cells of the intestinal mucosa meet their nutrient requirements first. It also seems likely that intestinal tissue is simultaneously acting to defend the organism against amino acid toxicity as one might infer from evidence of the substantial metabolism of indispensable amino acids in this tissue (1–5). The use of dietary essential amino acids as energy fuels (6–8), before getting anywhere near their systemic sites of utilization, otherwise makes little sense.

Regardless of the underlying logic, the fact of the matter is that the net portal appearance of amino acids is distinct in quantity and proportions from the amino acids disappearing from the lumen. Whatever the fate of amino acids extracted by the gut, the evidence clearly favors a significant role of the intestine in modulating the profile of amino acids delivered to the rest of the body (9,10). Intestinal metabolism has important implications for the ability to deal with large amounts of amino acids during both enteral and parenteral feeding. Under conditions of enteral feeding, the intestinal capacity for amino acid metabolism in the first pass greatly modifies the incoming amino acid mixture. The metabolism of dietary essential amino acids by the gut has a direct effect on their systemic availability. The ability to tolerate large oral doses of amino acids is a direct function of this capacity. Under conditions of parenteral feeding, the intestine atrophies dramatically, not only reducing...
the total amino acid requirements of the whole body but also creating a situation where first-pass metabolism is no longer contributing to balancing of the amino acid mixture provided.

**Intestinal protein synthesis and degradation**

The concept that gastrointestinal tissues constitute a large fraction of whole-body protein synthesis and turnover is well established. Rates of protein synthesis in intestinal mucosa are among the highest in the body and can be more than an order of magnitude greater than those observed in peripheral tissues such as skeletal muscle (11–13). The participation of protein synthesis (K_{syn}) and degradation (K_{deg}) in the regulation of gut protein balance and their modulation by specific nutrients are the focus of our work (14–16). The route of nutrient supply is important in the regulation of intestinal protein metabolism, because total parenteral nutrition, compared with enteral feeding, leads to profound atrophy and a large reduction of protein synthesis. We initially hypothesized that luminal exposure to specific amino acids or energy fuels would be required to stimulate intestinal protein synthesis. To test this, we developed an in situ experimental system that allowed controlled exposure of intestinal mucosa to nutrients systemically, luminally, or both.

Young piglets were food deprived overnight and then maintained under general anesthesia for up to 90 min, and four short jejunal segments within each piglet were cannulated in situ for luminal perfusion with solutions containing various nutrient mixtures. In our initial validation of the model, we compared K_{syn} in jejunal mucosa using the intravenous flooding dose technique with the administration of a comparable concentration and specific activity of ^{3}H-phenylalanine in a luminal perfusate (14). The routes of tracer administration and surgery and perfusion per se had no effect on mucosal K_{syn}. Furthermore, the four jejunal segments (within a piglet) had similar K_{syn} values (43 ± 2%/d). Because of the relatively small fraction of the small intestine implicated in the four segments, there was no detectable systemicaccumulation of any of the perfused nutrients. The system described allowed multiple comparisons within an animal and provided a robust model to study acute modulation of protein metabolism in intestinal mucosa by defined luminal stimuli.

When jejunal segments were randomly assigned to luminal perfusion with different nutrient mixtures, energy substrates (glucose, short-chain fatty acid mixtures, or β-hydroxybutyrate alone) had no effect on mucosal K_{syn} (15). Additionally, K_{syn} was not stimulated by a 30-mmol/L amino acid mixture similar in composition to jejunal digesta or 30 mmol/L glutamine. We then proceeded to examine the effects of systemic glucose and amino acid infusion on mucosal K_{syn} in piglets food deprived overnight (16). Intravenous infusion of glucose increased mucosal K_{syn} by 16% (i.e., a degree similar to that observed after refeeding food-deprived animals), whereas intravenous infusion of amino acids at levels reached during feeding in meal-fed piglets had no effect on K_{syn}. Our findings would appear to suggest that modulation of protein balance in the intestine in response to nutrients is attributable to anabolic stimulation of protein synthesis initiated by the systemic appearance of glucose, and possibly other factors that reflect nutrient availability systemically.

Our published work to date has also included parallel evaluations of mRNA levels of components of the ubiquitin-proteasome proteolytic pathway, including ubiquitin, 14-kDa ubiquitin conjugating enzyme, and the C9 subunit of the proteasome, demonstrating the sensitivity of components of the ATP-ubiquitin proteolytic pathway to acute regulation by nutrients (15,16). Levels of these mRNA were reduced by intravenous or luminal amino acid infusion. This effect was greatest (−50%) when the highest tissue concentrations of amino acids were achieved by the simultaneous infusion of amino acids by both routes. Because amino acids appear to be a key factor required to reduce expression of genes connected with proteolysis, we have sought to establish direct measures of protein degradation within our experimental system by an adaptation of the “pulse-chase” approach (V. E. Baracos, unpublished results). Four jejunal segments in this case are subjected to a pulse of ^{3}H-phenylalanine, followed by chase with 2 mM phenylalanine for 90, 60, 45, and 20 min, respectively. The protein-bound specific activity of phenylalanine is then determined and a log plot of this value against duration of chase is generated. In the presence of luminally perfused amino acids, the slope of this relationship was reduced by 50%, indicating a strong reduction in K_{deg} of mucosalproteins. Both protease mRNA and protein degradation appear to be strongly suppressed by amino acids.

The implications of the intestinal capture of amino acids for protein synthesis spans the range of visceral organ size and metabolic activity. Gut atrophy, on the order of 50% is reported during intravenous feeding along with a substantial reduction of K_{syn} (17) whereas gut hypertrophy and activation of protein synthesis can conspire to triple the total amount of protein synthesized within this organ system under conditions of maximal nutritional stimulation (18).

**Intestinal first-pass capture, transformation, and oxidation of amino acids**

Burrin and co-workers (7–9) instrumented young piglets with portal, arterial, and duodenal catheters and a portal blood flow probe, during conditions of diet infusion via the duodenum, for determination of the portal balance of amino acids. The animals also received duodenal and intravenous infusions of different amino acid tracers to measure the appearance across and the use of the tracer by the portal-drained viscera (PDV). Using these approaches, the proportion of dietary amino acid supply metabolized by the PDV can be discriminated. These authors suggest the presence of more or less extensive first-pass intestinal catabolism of dietary dispensable and indispensable amino acids in piglets, that on average, only 56% of the essential amino acid intake appeared in the portal blood (9). By contrast, the net portal balance (expressed as percentage of intake) of alanine (205%), tyrosine (167%), and arginine (137%) exceeded their intake. This group went on to study physiological adaptation of first-pass metabolism. When pigs were fed a high-protein diet, 84% of threonine was retained by the gut, and on a low-protein diet, all of the threonine was retained in the intestinal tissues (8).

The primary fate of indispensable amino acids is presumably protein synthesis; however, recent intriguing data demonstrated that catabolism dominates the first-pass utilization of these amino acids by the gut (8,9,19). Amino acids labeled with ^{13}C delivered luminally appear in part as ^{13}CO_{2} in first-pass metabolism and although this may be substantial for dispensable amino acids such as glutamate, it is not negligible for indispensable amino acids such as leucine. This seemingly wasteful oxidation of indispensable amino acids amounts to a small but significant proportion of dietary intake (1,8,20,21), but a large proportion of whole-body amino acid oxidation (8). In related studies, these authors have also demonstrated that the quality of dietary protein (22–24) and type of dietary carbohydrate (25) also influence first-pass amino acid catabolism. Although less studied to date, one would presume that...
stress or injury to the intestine (i.e., tissue injury, infection, antinutritional factors) may increase the extent and alter the pattern of first-pass metabolism. Stoll et al. (7,26) demonstrated that phenylalanine extraction was 50% higher in pigs raised outdoors, where there is greater exposure to pathogen challenges, than in a controlled environment indoors. Yu et al. (27) showed that a low-grade nematode infection increases total gastrointestinal tract leucine capture by 24% and gastrointestinal tract oxidative losses of leucine by 22–41% in sheep. In another study in pigs, the infusion of endotoxin led to enhanced intestinal catabolism of amino acids (28).

The gut deficient model for determination of amino acid requirements under conditions of enteral and parenteral feeding

Ball and co-workers have made extensive use of comparisons of parenterally fed piglets with orally fed piglets, to discriminate to what extent whole-body amino acid requirements are altered under conditions of extensive intestinal atrophy. The body of literature produced by this group regarding amino acid requirements during intravenous or intragastric feeding in piglets constitutes the available evidence on amino acid requirements with and without first-pass splanchnic metabolism. These authors combined intravenous or gastric feeding with the indicator amino acid oxidation technique to assess threonine requirements initially and thereby revealed a rather large differential between parenteral and enteral requirements for this amino acid. For example, the mean requirement for parenteral threonine intake was 0.19 g/kg/d, whereas the mean oral requirement was estimated to be 0.42 g/kg/d, a reduction of 55% (29). These data are concordant with those of Stoll et al. (9) who had also observed that the portal-drained visceral tissues extract 60% of dietary threonine, as determined by net portal balance and by labeled threonine extraction. This demand for threonine by the gut is probably reflected by its role in synthesis of the protective mucin secretions, which have a high threonine content (30).

The use of this “gut-deficient animal” has been extended to numerous other amino acids. The sulfur and branched-chain amino acids also are significantly utilized by the PDV, albeit not so greatly as threonine, whereas tryptophan, lysine, phenylalanine, and tyrosine utilization by the gut appears insignificant. In the presence or absence of dietary cysteine, the methionine requirements in parenterally fed piglets was 72% or 69% of the respective requirements in orally fed piglets (31). Using a diet with a fixed ratio of branched-chain amino acids (1:1.8:1.2, isoleucine:leucine:valine), these authors showed that the requirement for these in intravenously fed piglets was 56% of that in orally fed piglets (32). The apparent uptake of 44% of enterally fed branched-chain amino acids by the PDV tissues is a significant finding because these are generally accepted to be metabolized in skeletal muscle. In addition, the pattern of amino acids in the plasma of enterally fed piglets suggests that intestinal tissues prefer leucine to isoleucine or valine. Valine and isoleucine do not appear to be utilized by the gut to the same extent and are therefore being passed more extensively to the systemic circulation. By contrast, the tryptophan requirements of parenterally and orally fed piglets were not different when identical diets were employed (33). This result suggests that the intestinal use of tryptophan for protein synthesis and/or for oxidation does not have a significant impact on whole-body requirements, or that intestinal protein does not contain very much of this amino acid. These authors also suggest modest utilization of lysine, phenylalanine, and tyrosine (34,35) by the intestinal tissues.

The more extensive evolution of these experimental approaches will result in a full definition of the oral and intravenous amino acid requirements in a spectrum of developmental stages, and physiological and pathological states. These kinds of determinations will be of particular interest in situations of transition from oral to intravenous feeding and vice versa.

Conclusion and implications for the potential toxicity of amino acids. Collectively, the approaches described here allow an estimation of the qualitative and quantitative aspects of intestinal capture and release of amino acids. Although data at present are only available for the newborn piglet, the application of these approaches to different ages, and physiological, nutritional, and pathological states will allow us to more fully elucidate the impact of intestinal metabolism on the supply of amino acids during enteral and parenteral feeding. The models described here have to date only been used to evaluate intestinal metabolism of amino acids at levels of protein feeding at or near requirements, however, these could all be valuable to establish maximal gut capacity to deal with amino acid insults under different physiological and nutritional conditions. These amino acid insults or challenges would consist of the provision of purified amino acids substantially above the habitual range of ingestion. The downregulation of intestinal protein degradation by amino acids contributes to controlling amino acid entry into free pools from proteolysis in the function of the dietary supply. Our models can be used to identify the maximal response and the amino acids most potent in eliciting this change. Studies of net portal amino acid appearance suggest that under conditions of feeding at or near requirements little or none of certain amino acids appear systemically, whereas others pass through substantially. This approach has application in studies to define the upward limits of the adaptive regulation of intestinal amino acid catabolism in response to high amino acid doses. During oral feeding, it might be speculated that only relatively high rates of ingestion of amino acids, especially indispensable amino acids, might eventually challenge the capacity for amino acid catabolism in the PDV and liver to an extent that would result in overt toxicity. During parenteral feeding there is inherently more risk of amino acid toxicity because the participation of the gut in deflecting the systemic appearance of large quantities of amino acids is largely lost. Comparisons of animals adapted to orally and intravenous feeding under conditions of amino acid challenge would allow a differential description of maximal amino acid tolerances under these feeding paradigms.

LITERATURE CITED

INTESTINAL AMINO ACID METABOLISM


