Biologically Active Dietary Peptides

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Abstract: A large variety of peptides are generated in the gut lumen during normal digestion of dietary proteins. Large quantities of small peptides (ie. dipeptides and tripeptides) are absorbed through the gut mucosa and represent the primary mechanism for absorption of dietary nitrogen. However, larger peptide fragments are also absorbed with absorption decreasing with increasing chain length. Many of these dietary peptides have been shown to have biologic activity and many are active in microgram quantities. These peptides may modulate neural, endocrine, and immune function. In this report, we review normal protein digestion and absorption. We then discuss the biological actions of the amino acids arginine and glutamine and the biologic actions of a variety of dietary derived peptides. We concentrate on the immune effects of these peptides. We illustrate the potency of dietary peptides with a discussion of the cardiovascular effects of carnosine. We also review biologic effects of different protein sources, which generate different peptide profiles during digestion. The implications of dietary peptides for modulation of disease are discussed.

INTRODUCTION

The gastrointestinal tract is the primary organ for digestion and absorption of nutrients. Many food components in the form of biologically active proteins, peptides, lipids, and other substances survive the digestive processes of the gastrointestinal tract and can enter the circulation. For example, a variety of peptides are generated during digestion of dietary proteins within the gastrointestinal tract. Although it was once through that all ingested protein was digested to amino acids prior to absorption, we now know that most ingested proteins are absorbed in the form of small peptides (primarily di- and tripeptides). In addition, small quantities of larger peptides and small proteins are also absorbed [1, 2]. Although these larger particles are absorbed in small quantities (i.e. a few micrograms to milligrams) and do not contribute to overall nitrogen balance, these particles can modulate cellular function through their biologic properties [3]. Peptides play major roles in neural, endocrine, and immune function. Thus, it is possible that absorbed intact peptides can exert biological activities during health and disease.

PROTEIN DIGESTION AND ABSORPTION

Dietary protein is partially digested in the stomach and small intestine via the successive actions of hydrochloric acid, pepsin, trypsin, and chymotrypsin [4]. Once in the peptide form, further hydrolysis occurs through the action of brush border and cytoplasmic peptidases. Although most protein is ingested as intact proteins, most nitrogen is absorbed from the gut in the peptide form, prior to complete digestion to amino acids (which requires many hours). It had been presumed that peptides entering the mucosal cells of the gut were wholly hydrolyzed in the gut and that only free amino acids leave the cells and pass into the mesenteric circulation or lymphatics. However, there is now substantial evidence that incompletely digested fragments of proteins (i.e. peptides) and even intact proteins can cross the small intestine and gain access to the systemic circulation in both health and disease [2]. Some diseases affect the integrity, permeability, and digestive capacity of the small intestine to an extent that permits larger quantities of macromolecules and peptides to enter the circulation. Interestingly, specific transporters for peptides have been identified in the gut [5, 6]. One such transporter, peptide transporter-1, is a hydrogen/peptide co-transporter and is found on the absorptive epithelium of small intestinal cells [5]. In contrast to hydrogen ion dependency, amino acid carriers show strong sodium dependent absorption. Peptides account for approximately 60-70% of luminal nitrogen in the proximal jejunum and 50% of luminal nitrogen in the distal small intestine. Following absorption into the enterocyte, most peptides are degraded to amino acids via cytoplasmic peptidases. However, some peptides escape further hydrolysis (resistant to luminal and enterocyte hydrolysis) and reach the blood stream intact [7-10] and may be excreted in the urine [11]. Interestingly, absorption of amino acids into the circulation is faster when presented in small peptide form compared to amino acid form (indicative of separate transport systems) [4, 12, 13].

The absorption of peptides and even protein from the intestinal tract of infants is well known. Examples include immunoglobulins, polypeptide growth factors, albumin, and ferritin. Many of the beneficial effects of colostrums (i.e. breast feeding) result from absorption of polypeptides. Although it is clear that absorption of polypeptides diminishes with age, it is also clear that biologically significant quantities of small peptides continue to be absorbed in adults. Many studies have demonstrated that small peptides cross the intestine and enter the circulation intact [10, 11, 14-17]. Matthews and colleagues first demonstrated intact peptide transport in the small intestine [18]. These investigators administered oral doses of glycine in the form of glycine, glycyl-glycine, or glycyl-glycyl-glycine and noted that a greater amount of glycine was absorbed from the di- and tripeptide forms. Human studies

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yielded similar results, supporting the intact absorption of peptides [14, 19]. Many other studies have also indicated faster absorption of amino acids from protein hydrolysates compared to amino acid mixtures.

Measurable amounts of peptide-bound amino acids appear in peripheral blood or urine after a protein rich meal [20]. The proportion of portal amino acids present in peptide form varies with the species: 15% in the guinea pig, 52% in the rat, 65% in sheep, and 78% in the cow [21, 22]. In humans, peptides comprise 10-15% of plasma amino acids [1]. Gardner demonstrated that 30% of amino nitrogen reaching the serosal surface of the gut was in the form of peptides [15]. Subsequently, these authors confirmed these findings using an in-vivo model [21]. Webb *et al.* [16] assayed for arterial-venous differences of amino acids and peptides across the gastrointestinal tract of calves following a mixed meal. These investigators reported that 70% of amino acids appearing in the portal blood were in the peptide fraction with molecular weights of 300-1500. Sleisenger *et al.* [23] reported significant quantities of intact peptides entering the mesenteric circulation during absorption of casein hydrolysates in the guinea pig. Chabance *et al.* [24] demonstrated the absorption of two biologically active peptides (i.e. kappa-caseinoglycopeptide and the N-terminal peptide of alpha s1-casein) in humans following ingestion of milk or yogurt.

Absorption of peptides from the gut is affected by chain length. Absorption decreases as chain length increases. To test the concept that biologically significant quantities of peptides/small proteins could be absorbed from the gut intact, we administered three polypeptides with known biologic activities to rats via the gut [25]. The peptides chosen for study were thyrotropin-releasing hormone (TRH, a tripeptide), luteinizing hormone releasing hormone (LHRH, a decapeptide), and insulin (a 51 amino acid polypeptide). Importantly, cleavage of these molecules at any site inactivates the molecules. We found that small quantities (<0.5mg of TRH and LHRH; 25mg insulin) of these polypeptides administered via the gastrointestinal tract produced biologic effects in the animals (i.e. release of thyroid stimulating hormone (TSH) or LH, hypoglycemia). We measured insulin levels in the blood following enteral administration of insulin. Levels increased significantly and were associated with hypoglycemia [25]. The amount of peptide required for producing a biologic effect increased as chain length increased. In support of these findings, oral TRH is capable of releasing TSH in humans [26, 27] and animals [28]. Oral administration of LRH (p-Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly) and an LRH metabolite (p-Glu-His-Trp) stimulates release of leuteinizing hormone [29, 30]. Danforth *et al.* [31] also documented intestinal absorption of the insulin molecule in the rat. Absorption of insulin can be augmented through use of protease inhibitors and hypertonic luminal contents [32]. In addition, Warshaw *et al.* [33] documented absorption of other macromolecules such as albumin from the gastrointestinal tract. Hemmings *et al.* [34, 35] studied peptide absorption by feeding radiolabeled gliadin to rats. Using immuno-detection techniques, he demonstrated the presence of radiolabeled peptides in blood and tissues (including brain) of the animals. Bloch *et al.* [36] administered radiolabeled albumin peptides into rat jejunum. They were able to detect nanogram amounts (up to 910ng/ml) of the albumin peptides in mesenteric blood following administration of 500 mg albumin peptides.

A variety of diseases affect protein absorption from the gut lumen (i.e. shock, sepsis, pancreatic insufficiency, inflammatory bowel disease, celiac disease). In many of these diseases, peptide absorption is better maintained than is amino acid absorption. For example, amino acid absorption is more affected in patients with celiac disease than is peptide absorption [37, 38]. Nitrogen retention is significantly higher in patients with Crohn's disease [39] receiving peptide-based diets compared to amino acid based diets. In addition, peptides are better absorbed than amino acids in patients with pancreatic insufficiency [40]. Patients with cystinuria and Hartnup's disease have selective disorders of amino acid transport. These patients do not develop protein malnutrition due to preserved peptide transport [12]. Interestingly, genetic defects related to amino acid transport are known (i.e. are not lethal), but there are no known genetic diseases of peptide transport (presumably these are lethal). A variety of diseases (i.e. sepsis, inflammatory bowel disease, and cancer), also affects permeability of the gastrointestinal tract. Permeability of the mucosa may be increased in patients with systemic diseases and in the presence of hypertonic luminal contents [32, 41]. Increased permeability may permit absorption of larger polypeptides.

It is clear that intact peptides generated from dietary proteins during digestion can reach the circulation intact and may have systemic effects [14-16, 20-28, 31-36]. The extent to which peptides reach the circulation depends upon the digestibility of the dietary protein and peptides, the digestive capacity of pancreatic and intestinal proteases (which may be diminished in patients with inflammatory bowel disease, pancreatitis, following chemotherapy or radiotherapy, and sepsis), and the permeability of the mucosa. The digestibility of protein depends upon its amino acid composition. For example, propyl bonds, the presence of blocked terminal residues including pyroglutamate, the presence of unusual amino acids such as beta-alanine, or unusual bonds (i.e. gamma-glutamyl or beta-aspartyl) or intermolecular bonds (i.e. protein-carbohydrate) all decrease susceptibility to digestion [2].

BIOLOGIC ACTIONS OF AMINO ACIDS

Amino acids possess biological activities in addition to their role as substrate for protein synthesis. Examples include arginine [42] and glutamine [43]. Arginine stimulates the release of growth hormone and prolactin, insulin and glucagon secretion, and serves as a substrate for production of nitric oxide. NO is a potent vasodilator and participates in the regulation of vascular tone and tissue blood flow. NO modulates expression of adhesion molecules, tissue factor, and cytokines. Arginine has a variety of effects upon immune function [44-46]. It enhances T-lymphocyte mediated functions, stimulates replication of thymic lymphocytes, increases release of interleukin-2 from stimulated T-lymphocytes, and increases lymphocyte responses to mitogens. Arginine also improves nitrogen retention and enhances wound healing [42, 44].

Glutamine is an important energy source for replicating cells (particularly those of the gut and immune system) and is involved in nitrogen transfer reactions [43]. It modulates gut integrity. Glutamine deficiency is associated with gut atrophy, loss of the gut barrier, and increased infections. Recent clinical studies suggest that glutamine supplementation decreases infections and improves outcome in critically ill and trauma/surgical patients [47, 48]. In addition, cysteine is a major component of glutathione, an important antioxidant compound.

BIOLOGIC ACTIONS OF DIETARY PEPTIDES

Biologically active proteins and peptides in the diet can affect cellular and organ function by binding to mucosal receptors and triggering second messenger production in the gastrointestinal tract or may be absorbed into the circulation and affect cells throughout the body [3, 14, 25, 49]. A full discussion of bioactive dietary peptides is beyond the scope of this review. There are numerous proteins in the diet and millions of peptides generated from these proteins during normal digestion. Importantly, many dietary peptides are biologically so potent that even small amounts entering the circulation could have major pathophysiological significance. These peptides may produce their affects in the body at concentrations of micrograms to milligrams per ml. Since human adults absorb approximately 100 grams of protein per day (i.e. 1-1.5g/Kg), only a small fraction of ingested protein need be absorbed to produce systemic effects. A few of these peptides will be presented to illustrate the biologic potential of dietary peptides.

Human colostrums and breast milk are usually the first source of nutrition for the infant. These foods contain many biologically active substances such as immunoglobulins, lactoferrin, and growth factors [50-53]. Two major groups of biologically active dairy proteins include those with antimicrobial actions and those with growth factor activity. The antimicrobial proteins include immunoglobulins, lactoferrin, lactoperoxidase, and lysozyme [52-55]. The growth factors include insulin-like growth factor-1 (IGF-1), transforming growth factor-beta, and related peptides. Breakdown products of casein with biological activity are also liberated during milk processing. Two examples include glycomacropeptide which has antimicrobial effects [56, 57] and casomorphin, which has endorphin-like effects [58, 59]. Milk also contains parathyroid hormone related peptide (PTHrP). This peptide is felt to play a role in mineral metabolism in the body, particularly during the neonatal period [60].

Investigators have reported that digestion of a variety of dietary proteins, such as casein and wheat gluten, releases peptides with opiate-like activity in both receptor and bioassays [61]. These compounds were named exorphins [62, 63] in contrast to endogenously produced opioids called endorphins. The peptides responsible for the opioid properties of beta-casein hydrolysates (beta-casein is a component of milk and used in many nutritional formulas) have been isolated and sequenced [64, 65]. Their N-terminal amino acid sequence is Tyr-Pro-Phe-Pro. This sequence differs from that of endorphins (i.e. enkephalins, beta-endorphins, dynorphin), which have the sequence Tyr-Gly-Gly-Phe. The beta-casomorphins have been found to produce naloxone-reversible analgesia following intracerebroventricular administration. However, we were unable to detect analgesia following enteral administration (using hot plate and tail flick). On the other hand, beta-casomorphin has been found to modulate gut permeability and motility. These peptides bind to gut luminal receptors and act as exogenous regulators of gastrointestinal motility, gut permeability, and gut hormone release [62, 63, 66]. Beta-casomorphin (Tyr-Pro-Phe-Pro-Gly-Pro-Ile) decreases small bowel electrical activity, enhances sodium and chloride absorption, stimulates mucin secretion, and modulates small bowel permeability [66-68]. Schusdiarra et al.[69] administered digested gluten into the stomach and noted a more rapid rise in peripheral vein insulin and glucagon levels than when an equivalent amount of undigested protein was administered. Additional studies suggested that the effect was related to activation of opiate receptors by exorphins. In addition, Rogers *et al.* [70] documented absorption of the tetrapeptide, L-Tyr-D-Ala-Gly-L-Phe, which had opiate-like activity as measured by bioassay.

A number of dietary peptides modulate immune function. Examples include peptides generated during digestion of casein [71] and soy proteins [72]. Casein peptides have been shown to stimulate phagocytic activity of murine and human macrophages and to protect against Klebsiella pneumonia infection in mice [71, 73]. A hexapeptide obtained from casein enzymatic digestion was purified. This peptide (i.e. Val-Glu-Pro-Ile-Pro-Tyr) stimulates phagocytosis by murine peritoneal macrophages and protects against bacterial infection [73, 74]. Two immunostimulating peptides obtained from human caseins (i.e. Val-Glu-Pro-Ile-Pro-Tyr, Gly-Leu-Phe) stimulate binding and phagocytosis in human-macrophages [73, 75]. These two peptides have been shown to bind specifically (i.e. via receptors) to human phagocytic cells [73]. Specific soy derived peptides stimulated the release of tumor necrosis factor and mononuclear cell phagocytosis [72]. Although specific peptides were not isolated, Bounous *et al.* showed that lactalbumin hydrolysates improved immune function and outcome from infection [76-78]. Methionine-enkephalin (Met-enkephalin) is a pentapeptide that can alter antibody responses, delayed cutaneous hypersensitivity responses, and allograft rejection [79, 80]. Met-enkephalin may alter age-related decline in immune function [80]. This peptide may be generated during protein digestion. The tripeptide arginine-glycine-aspartate is a component of cell attachment sites for microbes. This peptide can antagonize adherence of Candida albicans to subendothelial binding sites [81].

A peptide with thyrotropin-releasing hormone (TRH) immunocharacteristics has been isolated from alfalfa [82, 83]. This peptide stimulates the release of thyroid stimulating hormone (TSH) but also inhibits release of prolactin. TRH is absorbed intact through the intestine [25, 26, 28]. Interestingly, TRH is distributed throughout the gastrointestinal tract [84] and modulates gastric acid secretion, pancreatic secretion, and gastrointestinal motility. Many common foods and nutritional supplements contain the bioactive peptide, cyclo (His-Pro) [85, 86]. Cyclo (His-Pro) is the active metabolite of TRH and is responsible for stimulating the secretion of TSH. Cyclo (His Pro) has also

been reported to modulate food intake (decreases appetite) [87].

A substance with LHRH activity has been found in leaves of oats [2, 88]. LHRH is active when administered orally [25, 29, 30]. The dipeptide histidine-methionine stimulates release of growth hormone in some acromegalics [89], suggesting the presence of pituitary receptors for the peptide in growth hormone producing tumors. The silkworm protein, sericin, has been reported to inhibit lipid peroxidation and tyrosine kinase activity [90]. When fed to mice, sericin inhibited development of colon cancer induced by 1,2-dimethylhydrazine [91].

In our laboratory, we discovered and evaluated the cardiovascular actions of carnosine (beta-alanine-histidine), a dipeptide found in meat, poultry, and fish [92, 93]. Carnosine is the primary intracellular peptide in the body and is found in high concentrations in muscle tissue. It has a variety of biologic properties, which include modulation of intracellular pH, antioxidant properties, and precursor for histidine. We discovered that carnosine possessed intrinsic inotropic properties in isolated hearts [94]. The peptide also vasodilated arteries [95]. The effect was highly dependent upon both constituent amino acids, beta-alanine and histidine. We found that carnosine increased free intracellular calcium levels in cardiac cells and that its effects were mediated via modulation of the release of calcium from the sarcoplasmic reticulum in cardiac cells [94] and calcium release from the endoplasmic reticulum in skeletal muscle cells. In aortic strips, we found that relaxation was dependent upon the generation of cyclic GMP [95]. We subsequently, administered carnosine to dogs and found dose-dependent vasodilation. Finally, carnosine was administered orally to humans and its affects upon cardiac output were assessed. Carnosine was found to significantly elevate cardiac output by 30% and its actions correlated with levels of the compound in the circulation (Dr. Pamela Roberts, personal communication). This is the first report of a dietary peptide altering cardiac function in humans.

Due to its vasodilating properties, the effect of carnosine on blood flow to the intestinal tract in an animal model of gut vasoconstriction using intravenous infusions of vasopressin was studied. Carnosine antagonized the vasoconstrictive effects of vasopressin on blood flow [96]. We have also found that carnosine improves wound healing in animals [97].

Some foods contain peptides, which antagonize the actions of angiotensin. For example, Astawan *et al.* [98] studied pepsin digests of tuna and found that it contained peptides, which inhibited angiotensin I-converting enzyme. The hydrolysate decreased the blood pressure of rats when administered orally. The investigators identified 4 specific peptides with angiotensin I converting enzyme inhibitory activity. The amino acid sequences of the peptides were Val-Ala-Trp-Lys-Leu, Trp-Ser-Lys-Val-Val-Leu, Ser-Lys-Val-Pro-Pro, and Cys-Trp-Leu-Pro-Val-Tyr. Milk (particularly fermented milk) contains peptides with angiotensin-I converting enzyme (ACE) inhibitory activity [99-101]. Nakamura *et al.* [101] isolated two tripeptides (i.e. Val-Pro-Pro, Ile-Pro-Pro) with ACE inhibitory activity from sour milk. ACE inhibitory peptides were found in the aorta of experimental animals after oral administration [102], providing evidence for enteral absorption. Fermented milk has been shown to lower blood pressure in humans [103, 104].

Vasopressin is a nonapeptide with an amidated C-terminus and has been shown to have antidiuretic activity when administered orally [105]. Desmopressin, a vasopressin analog that lacks vasoconstrictive activity, is also active orally and has been used to treat patients with bed-wetting.

The gut serves as a barrier to microbes and toxins as well as the primary absorptive organ. Interestingly, amino acid based diets (lacking dietary peptides) are associated with atrophy of the gastrointestinal tract and diminished function of the gut barrier [106-111]. These findings suggest that dietary peptides maintain gut structure and function. Mechanisms include direct stimulation of gut growth factors as well as stimulation of gut trophic hormones [13, 106]. Liver function during shock is also better preserved with peptide vs amino acid based diets [112, 113]. Non-peptide based diets have been associated with impaired immune function. For example, macrophage tumor cytotoxicity is impaired [109]. Non-peptide based diets are also associated with higher mortality following hemorrhagic and septic shock [110, 112-114]. A number of studies have demonstrated improved nitrogen utilization and growth in animals receiving peptide-generating diets versus amino acid based diets [106, 115-118]. These effects appear to result from the combination of better nitrogen utilization and higher trophic hormone levels.

BIOLOGIC ACTIONS OF DIFFERENT PROTEIN SOURCES

Studies of protein sources also support the concept that peptides generated during digestion modulate biologic functions. If the concept is correct, one would predict that diets containing the constituent amino acids of a protein would produce different effects than the protein itself or its hydrolyzed products. Protein balance in animals is better on peptide generating diets (intact and hydrolyzed protein) compared to amino acid diets. The improved balance reflects a decrease in catabolism (decreased nitrogen excretion) and improved synthesis. Wound healing is also improved with peptide vs. amino acid based diets, reflecting improved protein synthesis. Synthesis of visceral proteins such as prealbumin, transferring and albumin are increased on peptide vs. amino acid based diets. Sitren et al [119] assessed outcome in methotrexate treated mice fed diets containing amino acids or peptides. Mortality was significantly higher on the amino acid based diet compared with peptide generating diets (i.e. hydrolyzed or intact protein). In radiation-injury studies, animals fed protein hydrolysates survived better than animals fed intact protein diets [120-122]. Protein digestion (generation of luminal peptides) is impaired after radiation injury.

One also hypothesizes that different proteins, which generate different peptides during digestion, produce different biologic actions. For example, the immune responses of mice fed lactalbumin hydrolysates are significantly greater than the immune responses of mice fed casein hydrolysates [123]. Immune response is also greater

in mice fed diets containing casein vs. legume protein [124]. Blood pressure is lower in individuals consuming vegetable protein vs. meat proteins [125]. Small intestinal transit time is enhanced with soy protein compared to casein [126]. Glomerular filtration rate and renal plasma flow are significantly higher in humans receiving animal vs. vegetable diets [127]. Sodium and water absorption are stimulated with casein and lactalbumin but not fish protein hydrolysates [128]. This effect is related to the concentration of the peptides.

Sitren *et al.* [119] assessed stool consistency and mortality after methotrexate administration to mice fed with casein or soy based diets. Diarrhea occurred in 60% of the casein group but none of the soy fed animals. The soy fed animals maintained better body weight and had higher survivals compared to the casein fed animals (60% vs. 20%). Recently, we investigated the effects of fish, casein, and *whey* proteins on intestinal fluid secretion induced by cholera toxin. Fish peptides inhibited secretion while casein and whey had no effects on secretion. Interestingly, fish protein has been associated with reduced diarrhea in some patients with inflammatory bowel disease.

There is abundant evidence that a variety of macromolecules (especially proteins) can be absorbed across the gastrointestinal tract. A dramatic example is the absorption of botulinum toxin, which is so potent that a few nanograms is lethal [129]. Other examples include immunoglobulins, insulin, albumin, and ferritin.

Biologically active proteins may also be consumed in the form of lactic acid bacteria and related microorganisms (so called "probiotics"). Common features of these bacteria are resistance to acid and bile degradation, which enable survival of the organisms through the stomach and small intestine, ability to adhere to mucosal epithelial cells and mucous allowing colonization of the gastrointestinal tract, and ability to secrete a number of biologically active peptides, many with antimicrobial properties. These organisms have been useful in treating diarrhea [130]. Studies also suggest that they can augment immune responses [131-135] and reduce carcinogenic transformation in the gut [136]. Probiotic bacteria have been shown to induce cytokines such as interferon-gamma, interleukins-1 beta, and tumor necrosis factor-alpha [137] and stimulate immunoglobulin production [131]. Antitumor activity may result from production of glycopeptides [138].

SUMMARY

It is now known that biologically significant amounts of dietary peptides and proteins cross the intestine and enter the circulation in healthy patients and patients with various diseases. We believe that peptides generated in the diet during digestion can modulate numerous biologic processes in the body. Peptides are absorbed intact and can affect cellular, neurologic, and hormonal responses. Clearly, we have known for many years that small peptides represent important signaling compounds in the body. Thus, the type of nitrogen source (i.e. amino acid vs. protein) and type of protein in the diet as well as the digestive and permeability characteristics of the gastrointestinal tract may modulate disease processes. Further investigation of dietary bioactive peptides is warranted and may represent important nutritional therapies for a variety of disease processes.

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