Regulation of expression of the intestinal oligopeptide transporter (Pept-1) in health and disease

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Adibi, Siamak A. Regulation of expression of the intestinal oligopeptide transporter (Pept-1) in health and disease. Am J Physiol Gastrointest Liver Physiol 285: G779–G788, 2003; 10.1152/ajpgi.00056.2003.—The abundance of the oligopeptide transporter (Pept-1) in the brush-border membrane of the intestinal epithelium is the central mechanism for regulation of transport of products of protein digestion (dipeptides and tripeptides) and peptidomimetic drugs (for example, β-lactam antibiotics). Within the past few years, there has been substantial progress in identifying the factors controlling this regulation and the mechanisms of their actions. The purpose of this report is to review this progress. The studies of individual substrates and hormones in a human intestinal cell line (Caco-2) have shown that dipeptides, certain amino acids, insulin, and leptin increase and epidermal growth factor and triiodothyronine decrease the membrane population of Pept-1. In the case of dipeptides, epidermal growth factor, and thyroid hormone, there are parallel changes in the gene expression brought about by alteration of transcription and/or stability of Pept-1 mRNA. In contrast, the treatment with insulin and leptin does not induce any alteration in the Pept-1 gene expression, and the mechanism of increased protein expression appears to be increased trafficking from a preformed cytoplasmic pool to the apical membrane. In vivo studies in rats have shown modulation of protein and gene expressions of the intestinal oligopeptide transporter during the day and during development and in nutritional and metabolic alterations, such as high-protein diet, fasting, and diabetes. Patients with intestinal diseases, such as ulcerative colitis, Crohn’s disease, and short-bowel syndrome, may have induction of the Pept-1 expression in their colon. Finally, pharmacological studies have shown that the expression of Pept-1 can be upregulated by agents such as 5 fluorouracil and downregulated by agents such as cyclosporine. In conclusion, the above studies have produced a wealth of new information on regulation of a key transporter in the intestine. This information may have useful applications in nutritional and pharmacological treatments, for example, in diabetic patients needing enteral nutrition or in ulcerative colitis patients needing the suppression of the intestinal inflammation.

drug therapy; gene expression; intestinal absorption; protein nutrition

The small intestine can be considered as the organ of nutrition because its main function is to assimilate the ingested foods. This assimilation provides the nutritional requirements for remaining healthy. Among these requirements is a daily supply of amino acids. By far the major source of amino acids is the protein constituents of plants and animals. Soon after the discovery of protein in the 19th century, its absorption became a topic of great interest among the life scientists. For a long time, it was widely believed that proteins are completely hydrolyzed in the gut lumen and then are absorbed as free amino acids. About 30 years ago, studies of protein digestion in the human small intestine revealed that, in contrast to the general belief, the main products of protein digestion in the gut lumen are not amino acids but are dipeptides and tripeptides (3). Subsequent studies of absorption of these peptides in the intestines of human volunteers led to the suggestion of the existence of a transport system for absorption of dipeptides and tripeptides (1, 4, 5). This suggestion, which was supported by the studies in the intestine of experimental animals (30), was recently validated by the cloning of the intestinal oligopeptide transporter in the intestines of man and experimental animals (11, 17, 18, 28, 44).

Physiological and molecular studies have shown that the intestinal oligopeptide transporter, designated Pept-1, is the exclusive oligopeptide transporter of the brush-border membrane of the intestinal mucosa (2).
Although the molecular structure, driving force, substrate specificity, and function of Pept-1 have already been previously reviewed (2, 16, 31), it is pertinent to underscore some of its unusual features. For example, in contrast to other transporters, the oligopeptide transporter has an enormous range of substrates. It includes 400 dipeptides and 8,000 tripeptides that could be produced from the digestion of dietary and body proteins and a wide range of drugs, which have dipeptide- and tripeptidilike structures, such as β-lactam antibiotics and angiotensin-converting enzyme inhibitors. Another unusual feature is its dependency on proton gradient for its uphill transport, whereas other transporters commonly depend on an Na\(^+/H^+\) gradient. In fact, Pept-1 is an H\(^+/H^+\) peptide cotransporter and belongs to a family of peptide transporters found in all species from bacteria to humans (16, 31).

Shortly after the discovery of intestinal absorption of oligopeptides, it was shown that this absorption is affected by nutritional and metabolic alterations and diseases affecting the small intestine (for a review, see Ref. 30). However, these studies could not provide insight into mechanism of any of these alterations because a multitude of processes are involved in peptide absorption. The well-established processes include the following (Fig. 1): 1) an Na\(^+/H^+\) exchanger located in the brush-border membrane that maintains an intracellular alkaline pH, 2) an Na\(^+-K^+\)-adenosine phosphatase (ATPase) located in the basolateral membrane that maintains an inside negative membrane potential, and 3) several cytoplasmic peptidases that prevent intracellular accumulation of absorbed peptides. These enzymes convert most of the absorbed oligopeptides to amino acids that are either used by the absorbing cells or are released into the portal circulation via the amino acid transporters located on the basolateral membrane of these cells. The oligopeptides that escape hydrolysis by the cytoplasmic peptidases are transported across the basolateral membrane into the portal circulation by a peptide transporter that appears to be different from Pept-1 (2, 31). According to Fig. 1, the oligopeptide transport could be regulated by alteration in activity or abundance of Pept-1, Na\(^+/H^+\) exchanger, Na\(^+-K^+\) ATPase, cytoplasmic peptidases, and basolateral oligopeptide transporter. This review will focus on regulation of Pept-1 expression, because it is the central regulating mechanism and, moreover, as yet, very little information is available on the role of other processes involved in the regulation of peptide transport. There is no doubt that the other processes, besides Pept-1, are involved in regulation. This is evidenced by the recent demonstration that inhibition of the Na\(^+/H^+\) exchanger results in decreased peptide absorption (53).

This is the first review in which the purpose is mainly to focus on recent advances in regulation of Pept-1 expression. The review is organized into three parts. The first part covers the physiological factors that have been identified to regulate the expression of Pept-1. The second part discusses the regulation in pathological conditions. The third part enumerates the pharmacological agents that have been shown to affect the expression of Pept-1.

REGULATION IN HEALTH

Substrates

A possible regulator of functional and/or protein expression of any transporter is its own substrates. Therefore, a relevant question is whether this is true in case of Pept-1. Two studies (50, 55), which independently investigated this question, provided firm evidence for this possibility. These studies required use of a Pept-1-expressing intestinal cell line that would allow investigation of the direct effect of a single oligopeptide without involvement of other factors such as...
hormones. This requirement was met with the selection of Caco-2 cells (a human intestinal cell line) as the model. Caco-2 cells in culture differentiate into polarized cell monolayers with microvilli on the apical membrane containing the enzymes and transporters, such as Pept-1, normally found in the human intestinal brush-border membrane. In one of the above studies (50), it was found that previous exposure of Caco-2 cells to glycylsarcosine (10 mM Gly-Sar) for 24 h increased the \( V_{\text{max}} \) of glycylglutamine (Gly-Gln) transport by twofold without any significant change in its \( K_m \). These data suggested an increase in the population of the peptide transporter in the apical membrane of these cells. Indeed, the Western blot analysis of the peptide transporter in the apical membrane of these cells showed more than a twofold increase in the protein expression of Pept-1.

After 24 h of incubation of Caco-2 cells with Gly-Sar, there could be some hydrolysis of this peptide. Therefore, it was possible that the stimulation in peptide transport was not the function of the dipeptide but the function of its constituent amino acids. This possibility was eliminated by the result of an experiment substituting Gly-Sar with a corresponding mixture of free glycine plus sarcosine. This experiment showed no stimulation in transport when this mixture was used (50).

Because it took 24 h for Gly-Sar to stimulate dipeptide transport, it was hypothesized that the mechanism of increase in the population of Pept-1 in the brush-border membrane of intestinal mucosa cells is by an increase in the synthesis of Pept-1 in the endoplasmic reticulum. To investigate this hypothesis, the following strategy was used. The insertion of the newly synthesized Pept-1 into the membrane requires processing by the trans-Golgi network (TGN). A preliminary experiment showed that brefeldin treatment of Caco-2 cells resulted in the disappearance of Golgi cisternae without any effect on other ultrastructures of these cells (50). This observation was used to investigate whether the disruption of the TGN prevented the stimulation in dipeptide transport by Caco-2 cells treated with Gly-Sar. The result showed that, indeed, the entire stimulation in dipeptide transport by Gly-Sar was abolished by this treatment. Therefore, the increased synthesis appeared to be responsible for the increased population of Pept-1 in the apical membrane of Caco-2 cells treated with Gly-Sar. The increased synthesis of a protein usually requires the increased expression of its gene. Indeed, the Northern blot analysis showed a threefold increase in the Pept-1 mRNA in Caco-2 cells incubated with Gly-Sar (50).

Gly-Sar is not a typical dipeptide because it is not found in nature. It is commonly used as a substrate for measuring the functional expression of the oligopeptide transporters, because it is resistant to hydrolysis. This may lead to the question of whether the effect of Gly-Sar is representative of the natural dipeptides. This question was answered by the results of another study (55), which also investigated the possible regulation of Pept-1 expression by its own substrates. Unlike Gly-Sar, Gly-Gln is a natural dipeptide. It was found that previous exposure of Caco-2 cells for 3 days to 4 mM Gly-Gln (replacing 4 mM medium Gln) resulted in a near twofold increase in the \( V_{\text{max}} \) of Gly-Sar transport by these cells without any change in the \( K_m \). Furthermore, the study (55) showed that the Gly-Gln treatment increased by about twofold the cellular mRNA level and membrane protein expression of Pept-1.

From the results of the two studies summarized above, it can be concluded that dipeptides, whether natural or chemically synthesized, regulate their own transporter by regulating its gene expression. There are two possible mechanisms for increasing the gene expression. One is by increasing the stabilization of mRNA encoding the gene, and the other is by increasing the gene transcription. Two studies (47, 55) have investigated the involvements of these mechanisms in increasing the gene expression of Pept-1 in Caco-2 cells. In one study (55), the treatment of Caco-2 cells with Gly-Gln increased the half-life (an index of stabilization) of Pept-1 mRNA. Because the increase in half-life did not fully account for the increase in mRNA level, it was suggested that an increase in gene transcription must also be involved (55). Indeed, the other study (47) showed that incubation of Caco-2 cells with dipeptides such as Gly-Sar, Gly-Phe, Phe-Val, Lys-Phe, and Asp-Lys directly stimulated the transactivation of the Pept-1 gene promoter that regulates the transcription of this gene. Furthermore, this stimulation did not occur with single amino acids, such as glycine, aspartate, glutamate, valine, and alanine, but did occur with lysine, arginine, and phenylalanine (47). The molecular basis of these transactivations appeared to be the presence of the amino acid-responsive element in the promoter region of the Pept-1 gene (47).

The above studies were all done in vitro. Therefore, the question becomes whether there is substrate regulation of Pept-1 in vivo. Two studies (15, 47) investigated this question in rats that were fed varying amounts of protein. These studies provided circumstantial but not conclusive evidence for the oligopeptides regulating Pept-1, because dietary protein increases the concentration of amino acids and oligopeptides in the gut lumen and may also cause a wide range of other metabolic alterations. The first study (15) found that a high-protein diet (50%), compared with a low-protein diet (4%), fed for 14 days increased by 1.5- to twofold the level of Pept-1 mRNA in the middle and distal regions of the rat small intestine. The second study (47) confirmed this observation but showed that 3 days of treatment are sufficient to show the effect of dietary protein. In addition, the second study showed that the increase in the gene expression could be induced just by feeding rats a standard diet but supplemented with a single dipeptide (Gly-Phe) for 3 days (47). To conclude that these increases in the Pept-1 mRNA indicated increases in the population of the transporter in the brush-border membrane needed determination of the transport kinetics and protein expression of Pept-1 in rats fed the low- and high-protein
diets. These determinations were performed in the second study, and the results provided firm support for the above conclusion.

In summary, a high-protein meal increases the concentrations of dipeptides, tripeptides, and amino acids in the gut lumen. These products serve as signals for increasing the gene expression of Pept-1, leading to increased population of the peptide transporter in the brush-border membrane. The ultimate result is the increased peptide transport, which is a central feature of intestinal adaptation to high-protein diets (20).

**Insulin**

The cloning of the intestinal oligopeptide transporter (11, 17, 18, 28, 44) allowed prediction regarding its protein structure. These included putative sites for protein kinase C- and cAMP-dependent phosphorylation. Because these sites are involved in the action of certain hormones, it appeared that there could be hormonal regulation of Pept-1. The first hormone to be studied was insulin, because it is considered to be the key hormone in metabolic regulation, particularly in the transport of glucose and amino acids in muscles. It was found that insulin, when added in a physiological concentration to the incubation medium, greatly stimulated dipeptide transport by Caco-2 cells (52). The stimulation was rapid (within 60 min) and required binding of insulin to its membrane receptor, because preventing this binding abolished the stimulatory effect of insulin on dipeptide transport (52). Lastly, the stimulation in dipeptide transport appeared to be specific, because insulin did not have any effect on glucose transport by Caco-2 cells (29).

Studies of the mechanism of insulin stimulation showed the following (52): the kinetic analysis revealed no significant change in the $K_m$ of Gly-Gln transport, thus eliminating the intrinsic activation of the transporter as a mechanism. On the other hand, it revealed a near twofold increase in the maximal velocity of Gly-Gln uptake, which suggested an increase in the number of the transporter. This suggestion was verified by qualitative and quantitative Western blot analyses of membrane Pept-1. The study of the mechanism of the increased membrane population of Pept-1 showed that insulin effect persisted even when Golgi apparatus, which is involved in trafficking of newly synthesized Pept-1, was dismantled. Furthermore, insulin treatment had no significant effect on the gene expression of Pept-1. These observations suggested that insulin treatment did not increase the synthesis of Pept-1. On the other hand, there was complete elimination of insulin effect by disruption of microtubules involved in the trafficking of preformed Pept-1 (52). These results, taken together, show that insulin, when it binds to its receptor, stimulates dipeptide uptake by Caco-2 cells by increasing the membrane population of Pept-1. The mechanism appears to be increased translocation of this transporter from a preformed cytoplasmic pool and not de novo synthesis.

Insulin is not a hormone that can be found in the gut lumen. However, under in vivo conditions, the circulating insulin could regulate peptide transport by its binding to its receptors that are located on the basolateral membrane of the intestinal mucosal cells. This question was not investigated in the above study (52) but recently was investigated by another laboratory (38). It was found that previous exposure of the apical membrane of Caco-2 cells to insulin had no influence on apical uptake of Gly-Sar, but the exposure of the basolateral membrane to insulin in a dose-dependent manner significantly increased this uptake. Insulin, in a physiological concentration, applied for 60 min to the basolateral membrane, significantly increased the $V_{max}$ of Pept-1-mediated Gly-Sar transport without any change in the $K_m$. Other new information presented by this study (38) was that a change in the intracellular pH was not responsible for the effect of insulin.

In contrast to insulin, there are hormones that are secreted by the gastrointestinal glands into the gut lumen. These include EGF and leptin. EGF stimulates proliferation of epidermal cells and a variety of other epithelial and nonepithelial cells, and leptin regulates energy homeostasis by altering energy intake and expenditure. Recently, both of these hormones have been shown to regulate the expression of Pept-1.

**EGF**

Incubation of Caco-2 cells with a physiological concentration of EGF inhibited dipeptide transport (39). The inhibition was apparent after 5 days of incubation, but it took >15 days to become maximal. Although both apical and basolateral membranes of Caco-2 cells have receptors for EGF, only the exposure of basolateral receptors to EGF resulted in the inhibition of dipeptide transport. The studies of mechanism of inhibition showed ~60% decrease in the $V_{max}$ of dipeptide transport without any significant change in the $K_m$, suggesting a decrease in the number of transporter. This suggestion was verified with the Western blot analysis of Pept-1. This decrease in the population of Pept-1 appeared to be due to a decrease in its gene expression (39).

Because both EGF and insulin mediate their cellular effects through tyrosine-kinase receptors, the opposing effects of these hormones on regulation of Pept-1 expression appeared paradoxical. This paradox was resolved by an additional study (38) that investigated the effect of EGF on Pept-1 expression after Caco-2 cells were briefly (for 1 h) exposed to this hormone. The rationale for this study (38) included the results of previous studies that had shown the long-term treatment (several days or longer) of Caco-2 cells with EGF results in a decrease in a number of brush-border membrane proteins (14).

After 5 min of exposure of the basolateral membrane of Caco-2 cells to EGF, there was a dose-dependent increase in the apical uptake of Gly-Sar (38). The kinetic analysis showed an increase in the $V_{max}$ of
Pept-1-mediated transport of Gly-Sar without any change in the $K_m$. The short-term EGF treatment did not alter either the Pept-1 mRNA level or the intracellular pH (38). However, the actual mechanism of increased Pept-1 functional expression remained unclear.

**Leptin**

Leptin secreted by the stomach may reach the lumen of the small intestine and, therefore, may be a regulator of intestinal functions such as absorption. Indeed, studies in rat intestine showed the presence of leptin receptors with preponderance in the jejunum (12). Furthermore, in vivo perfusion studies in the rat jejunum showed that introduction of leptin into the perfusate specifically increased peptide absorption without any effect on fluxes of water, electrolytes, or glucose (12).

Because studies in Caco-2 cells revealed the presence of leptin receptors, these cells were used to investigate the mechanism of leptin stimulation of peptide absorption (12). Within 30 min, leptin increased the transport of Gly-Sar and cephalixin (a peptidic antibiotic) by Caco-2 cells. This increase in transport was observed when leptin was added to the apical side but not to the basolateral side of these cells. The kinetic analysis of this increased transport showed a 51% increase in the $V_{\text{max}}$ without any change in the $K_m$, thus eliminating the modification of intrinsic activity of Pept-1 as a mechanism. Further investigation of the mechanism of leptin action showed a 60% increase in the membrane Pept-1 protein and a 50% decrease in the intracellular Pept-1 protein when this hormone was added for 30 min to the apical compartment of Caco-2 cells. These alterations were not accompanied by any change in the cellular Pept-1 mRNA level. To investigate the mechanism of leptin effect on Pept-1 protein, Caco-2 cells were pretreated with brefeldin and colchicine. The rationale for these studies has already been described in the section on insulin. It was found that the leptin stimulation of Gly-Sar transport was not prevented by brefeldin pretreatment, but it was abolished with colchicine pretreatment. Therefore, it appears that insulin and leptin share a common mechanism for stimulating dipeptide transport in Caco-2 cells, namely by increasing the trafficking of Pept-1 from intracellular pool to the apical membrane.

**Thyroid Hormone**

The above hormones initiate their action by binding with their respective receptors on the cell membrane, but there are hormones, such as triiodothyronine (T$_3$), that enter the cell for initiating their action. Recently, it was reported (6) that pretreatment of Caco-2 cells with 100 nM T$_3$ for 4 days resulted in significant inhibition of Gly-Sar uptake. The effect appeared to be specific, because no inhibition of transport of any other nutrients was observed. The studies of the mechanism of inhibition showed the following (6): the kinetic analysis of the Gly-Sar uptake showed no change in the $K_m$, but a near twofold decrease in the $V_{\text{max}}$, suggesting a decrease in the membrane population of Pept-1. This suggestion was supported by the Western blot analysis of the membrane abundance of Pept-1. Because there was also a decrease in the gene expression, it was suggested that T$_3$ inhibition of dipeptide transport is due to a decrease in the transcription and/or stability of Pept-1 mRNA (6). However, as far as the effect on the transcription is concerned, there is no evidence for the presence of thyroid hormone-responsive element in the promoter region. Nevertheless, it is possible that after binding to its receptors on cell nucleus, T$_3$ could affect transcription by indirect effects.

**Diurnal Rhythm**

A number of metabolic processes, including the transport of some nutrients, has a diurnal rhythm. Recently, whether peptide transport has such a rhythm was investigated (42). The rats were allowed free access to water and standard laboratory chow, with a 12-h lighting schedule (0800–2000). Intestinal transport of Gly-Sar, measured by both in vivo and in vitro techniques, was found to be greater during the dark than during the light phase. To investigate a mechanism for this difference in transport, the population of Pept-1 in the duodenal brush-border membrane at various times of day was determined. The Western blot analysis showed that this population was highest at 0000 and lowest at 0800. The mechanism of this variation appeared to be transcriptional, because the levels of Pept-1 mRNA during the day showed the same pattern. Although the metabolic signal for this regulation is not known, the daily variation in the expression of peptide transporter appears to be related to eating habits of rats, which is mostly at night (42).

**Development**

A critical period in life is the development during peri- and postnatal periods. Studies performed over two decades ago showed that there is a burst of peptide transport activity in the intestine around birth that is not accompanied by any change in amino acid transport activity (24, 25). After the intestinal oligopeptide transporter was cloned, four laboratories (26, 34, 43, 46) studied aspects of the molecular expressions of Pept-1 during development. The first study (34) found the level of the intestinal Pept-1 mRNA to be dramatically increased in 10-day-old rats and then decreased reaching the adult level by the 28th day after birth. The second study (46) investigated intestinal Pept-1 mRNA and protein levels at regular intervals from 17 days before to 75 days after birth in rats. It was found that Pept-1 mRNA levels increased rapidly at birth in the duodenum, jejunum, and ileum and attained maximal levels by the 3rd to 5th day after birth. The levels then fell rapidly but rose transiently on the 24th day, about the time of weaning. The adult Pept-1 mRNA levels were ~25% of the levels observed on the first few days after birth. Essentially, a similar pattern of expression was also observed for the protein expression of Pept-1 (46). An unexpected finding was the detection of
a transient Pept-1 expression in the colon during the first few days after birth. Previous studies had found no Pept-1 expression in the colon, but the studies were all performed in adult rats. The third study (26), which determined the protein expression of Pept-1 in rat duodenum at the 18th day of gestation, birth, weaning (21 days after birth), and adulthood found it to be strongest at birth. The expressions at weaning and adulthood appeared similar but stronger than the one at 18th day of gestation. But, unlike the previous study (46), no second peak at weaning was found. The last study (43), which determined the proximal-distal distribution of Pept-1 expressions in the rat intestine from postnatal days 4 to 50, found no change during this period. In summary, these studies provide molecular basis for the earlier physiological observations on peptide transport activity during development. Additional studies are still needed to identify the inducer of Pept-1 gene expression during development.

REGULATION IN PATHOLOGICAL CONDITIONS

Fasting

A common nutritional condition is a brief fast in patients who become acutely ill. With this condition as a model of malnutrition, its effect on functional and molecular expressions of Pept-1 was investigated (51). Rats were fasted for 24 h before the jejunum was removed for the following studies. Fasting greatly increased the rate of Gly-Gln uptake by the brush-border membrane vesicles. The investigation of mechanism of this increase in transport by analysis of its kinetic constants showed that the $K_m$ was unaffected, whereas the $V_{max}$ was increased by twofold, suggesting an increase in the membrane population of Pept-1. This suggestion was verified by the Western blot analysis of Pept-1 located in the brush-border membrane (51). The mechanism of the increase in the population of the transporter appeared to be pretranslational, because fasting increased the gene expression of Pept-1 by several fold (51).

The report of an earlier study (45), using rings of everted hamster jejunum, that starvation for 3–4 days reduces the rate of Gly-Sar transport, raised the question of whether the increases in Pept-1 expressions shown above would persist if fasting is prolonged. Two laboratories (27, 41) studied the effect of 4 days of starvation on Pept-1 in rat intestine. The first study (41) showed that 4 days of starvation markedly increased the protein mass of Pept-1 in the jejunum as examined by immunoblotting and image analysis of immunofluorescence. The second study (27) showed that starvation for 4 days and semistarvation (half of daily food intake) for 10 days increased the Pept-1 mRNA and protein in the rat jejunum. Therefore, it appears that the upregulation of Pept-1 expression occurs whether fasting is brief or prolonged, and the difference between the earlier (45) and recent (27, 41, 51) studies appears to be methodological, for example, rings of everted intestine (45) vs. brush-border membrane vesicles (51).

Because all the above studies were performed in the jejunum, the question remained whether starvation affected the Pept-1 mRNA expression along the length of the small intestine. The most recent study (37) that investigated this question reported that the induction of Pept-1 mRNA expression occurs in all segments, but it is more pronounced in the middle to upper segments.

The fact that cell population of Pept-1 is increased in starvation may explain a paradox that appeared in the results of studies performed nearly two decades ago. Because starvation greatly reduces the absorptive surface of the intestine (19), it was expected that 14 days of starvation would reduce amino acid and peptide absorption in the jejunum of human volunteers. Indeed, the amino acid absorption was reduced, but unexpectedly, there was no significant change in peptide absorption (54). With the use of the present evidence, it could be explained that the reduction in the absorptive surface was counterbalanced with the increase in the cell population of the oligopeptide transporter, resulting in no change in peptide absorption.

In summary, fasting, whether brief or prolonged, increases the Pept-1 mRNA level, resulting in an increase in the population of the transporter in the brush-border membrane of the intestinal mucosa. The signal for this induction remains to be identified. The physiological significance may include counterbalancing the shortening of the absorptive surface during starvation.

DIABETES

As already discussed, studies in Caco-2 cells had shown that insulin upregulates Pept-1 (52). An implication of this finding is that there could be downregulation of Pept-1 in diabetes. To study this possibility, the activity of Pept-1 in the brush-border membrane vesicles prepared from the jejunum of diabetic rats deprived of insulin for 96 h was determined (22). In contrast to the above expectation, the activity was significantly increased. Kinetic studies showed that the ratio for this increased activity was not a change in $K_m$ but a significant increase in $V_{max}$. These kinetic findings suggested an increase in the number of transporters, which was verified by the Western blot analysis of Pept-1 in the brush-border membrane of jejunal mucosal cells. The mechanism of increased abundance of transporter appeared to be transnational, because there was a corresponding increase in the abundance of mRNA encoding Pept-1. Finally, increased stability, rather than increased transcription, appeared to be the mechanism of increased gene expression of Pept-1 in uncontrolled diabetes, because the gene transcription was not affected (22).

To investigate whether the effect of diabetes on Pept-1 was specific to the intestine or whether it affected other tissues, similar studies were performed on Pept-1 located in the brush-border membrane of renal tubules (22). The results showed that, as in the intestine, uncontrolled diabetes increased protein and...
mRNA expression of Pept-1 without affecting the rate of its gene transcription.

The above results indicate that, besides insulin, there are other systemic factor or factors that upregulate Pept-1. This is consistent with the demonstrated involvement of multiple factors in upregulation of Pept-1. Whatever the regulatory factor, the upregulation allows increased availability of substrates for the enhanced gluconeogenesis in diabetes.

**Intestinal Resection**

Intestinal resection is commonly used in treatment of diseases of the intestine. Studies in experimental animals have provided substantial evidence that an extensive resection of the proximal intestine causes mucosal hyperplasia in the distal small intestine and, to a lesser degree, in the colon accompanied with increased absorption of nutrients from these segments (40). Over two decades ago, investigation of these problems in rats showed that the increased absorption in the distal small intestine also includes dipeptides (23). Surprisingly, a recent investigation of Pept-1 mRNA levels in the ileum of rabbits who had undergone a small intestinal resection showed no significant change (7). Consistent with this observation, a subsequent study (57) also found no significant change, either in protein or mRNA expression of Pept-1 in the ileum of patients with short-bowel syndrome caused by resection of the proximal small intestine. However, it was found that the Pept-1 protein and mRNA levels were greatly increased in the colon of these patients (57). These expressions, which were faintly detectable in the normal subject, were increased by fivefold in patients with short-bowel syndrome. These results indicate that 1) the increased dipeptide absorption from the distal small intestine after resection of the proximal small intestine is not due to upregulation of Pept-1 and 2) there is a factor that induces Pept-1 expression in the colon, but its identity remains to be investigated.

**Intestinal Transplant**

Intestinal absorptive function has been reported to be reduced following small intestinal transplantation (56). As far as expression of Pept-1 is concerned, the study of the effect of transplant has produced conflicting results. There was an increase in protein expression of Pept-1 after syngeneic small intestinal transplantation in rats (36). Because there was no increase in Pept-1 mRNA level, it was suggested that this increase in the Pept-1 protein level was due to a decrease in protein degradation (36). On the other hand, there were decreases in Pept-1 protein and mRNA levels in rats subjected to allogeneic transplantation. Furthermore, these decreases were not prevented by treating the small bowel rejection. These studies indicate a complex regulation of Pept-1 expression after intestinal transplantation.

**Inflammatory Bowel Disease**

It has been reported that intestinal bacteria, such as *Escherichia coli*, secrete proinflammatory peptides. Among these peptides are n-formylated peptides. There is in vitro and in vivo evidence that Pept-1-mediated transport of n-formyl peptides, such as the tripeptide N-formylmethionyl-leucyl-phenylalanine, induces intestinal inflammation (13, 33). Indeed, a recent study (32) reported the induction of Pept-1 expression in the colon of patients with ulcerative colitis and Crohn’s disease. The factor inducing the Pept-1 expression in the colon of patients with inflammatory bowel disease remains unknown. One hypothesis could be that there is overproduction of the bacterial proinflammatory peptides in the colon of these patients, which, as substrates, induce the ectopic expression of Pept-1.

**Infection with Cryptosporidium Parvum**

Worldwide, *C. parvum* is a common cause of diarrhea in humans. In experimental animals, this infection causes villous atrophy and impairs (7) absorption of amino acids, glucose, and sodium. Most recently, there has been investigation of Pept-1 expression in the small intestine of suckling rats (8). In these animals, the infection reaches a peak, particularly in the ileum, 10–12 days after the intragastric administration of *C. parvum*, and there is spontaneous clearance after 21 days. Pept-1 mRNA analysis of the small intestine showed that the level was increased at the peak of infection and normalized after the spontaneous clearance of the parasite (8). Immunolocalization of Pept-1 showed that all along the small intestine, the protein expression was maintained during the infection. These studies (8) suggested that there is a transcriptional upregulation of Pept-1 during acute infection with *C. parvum*.

**REGULATION BY PHARMACOLOGICAL AGENTS**

**5-Fluorouracil**

Anticancer drugs, such as 5-fluorouracil, have deleterious effects on the intestinal mucosa, for example, causing cellular injury and reducing absorptive functions (49). There has been investigation of the effect of 5-fluorouracil on Pept-1 expression in the rat small intestine 3 days after an intragastric administration of this drug (49). There was no significant difference in Gly-Sar transport between brush-border membrane vesicles isolated from the treated and control rats. In contrast, the 5-fluorouracil-treated rats showed great reduction in amino acid and glucose transport. The immunoblot analysis of the small intestine revealed only a small decrease in the protein expression of Pept-1 but profound decreases in the protein expressions of other transporters. Studies of mRNA level after treatment showed the most dramatic difference between Pept-1 and other transporters. There was a greater than twofold increase in the gene expression of Pept-1, whereas there were great decreases in the gene expressions of other nutrient transporters. The results
suggested that the reason for the resistance of Pept-1 expression to the cellular injury caused by 5-fluoro-uracil is that this treatment increases the synthesis of Pept-1 (49). If this suggestion proved to be valid, 5-fluoro-uracil may be used as a pharmacological agent to upregulate Pept-1.

**Clonidine**

A study of in vivo absorption of β-lactam antibiotics led to the conclusion that their absorption is influenced by the nervous system (9). As already mentioned, the absorption of these peptidomimetic drugs is mediated by Pept-1 (9). Among the agents that were used in this study was clonidine. Clonidine is an agonist for α2-adrenergic receptors, and its administration to rats increased by twofold the intestinal absorption of β-lactam antibiotics. Because α2-adrenergic receptors are also present in the intestinal epithelial cells, it was possible that the clonidine directly altered the transport of these drugs. This possibility was investigated in a subsequent study (10) by determining the effect of clonidine on transport of cephalixin (a β-lactam antibiotic) by Caco-2 cells that were engineered to stably express α2A-adrenergic receptors at a density similar to that found in the mucosa. Addition of clonidine to the basolateral compartment of these specially cloned cells rapidly increased the transport of cephalixin by two-fold. This stimulatory effect of clonidine was not observed in the presence of Gly-Sar, indicating the involvement of Pept-1. Furthermore, the effect of clonidine was blocked by α2-antagonists, suggesting the involvement of α2-adrenergic receptors. Analysis of the kinetics of cephalixin transport showed that clonidine increased \( V_{max} \) without changing the \( K_m \), suggesting an increase in the number of membrane transporter. Because the effect of clonidine was quite rapid, the regulation at the level of transcription did not appear likely. On the other hand, microtubular integrity appeared necessary for the effect of clonidine, because treatment of Caco-2 cells with colchicine completely abolished the stimulatory effect. These data suggest that the clonidine increases the translocation of preformed cytoplasmic Pept-1 to the apical membrane of Caco-2 cells, resulting in increased transport activity, a mechanism similar to that described for the effect of insulin (52).

**Pentazocine**

The \( \sigma \)-ligand receptors may be involved in the pathogenesis of psychiatric disorders. These receptors, besides the central nervous system, are also found in nonneural cells, such as the gastrointestinal tract. There has been investigation of the regulation of Pept-1 by pentazocine, a selective \( \sigma \)-ligand, in Caco-2 cells (21). Apparently, \( \sigma \)-receptors are expressed in Caco-2 cells. It was found that after 2 h of treatment with pentazocine, Gly-Sar uptake was increased in a time-dependent manner. After 24 h of treatment, the \( V_{max} \) was increased by twofold without any change in the \( K_m \) of Gly-Sar transport. To elucidate whether this upregulation of Gly-Sar uptake could be attributed to a relative increase in Pept-1 synthesis, the level of Pept-1 mRNA was determined in the treated cells. The results showed that the \( \sigma \)-receptor ligand pentazocine, indeed, increased the level of Pept-1 mRNA. It was suggested that this increase leads to an increase in the density of the transporter protein in the cell membrane, which leads to an increase in peptide transport activity (21).

**Tacrolimus and Cyclosporin**

Immunosuppressive agents tacrolimus and cyclosporin may cause deleterious effects on organs such as the gastrointestinal tract. There has been investigation of the effects of these drugs on Gly-Sar transport in Caco-2 cells (35). It was found that each treatment reduced Pept-1 activity and transcellular transport of Gly-Sar from the apical to the basal side. Kinetic analysis showed that both treatments reduced \( V_{max} \) of Gly-Sar uptake without any effect on the \( K_m \). The mechanism of transport inhibition was not investigated, but apparently it did not include a decrease in the level of Pept-1 mRNA.

**LPS**

Bacterial endotoxemia, probably through increased release of proinflammatory cytokines, may alter intestinal function. An experiment was performed to investigate whether the expression of Pept-1 is affected in the small intestine of rats injected for 3 days with LPS *Escherichia coli* (48). LPS injection, either for 1, 2, or 3 days, decreased the abundance of Pept-1 mRNA in the jejunum and ileum. These decreases were not specific to Pept-1, because the mRNA expressions of other nutrient transporters were also decreased. The decrease in Pept-1 mRNA was accompanied with a similar decrease in the Pept-1 protein expression. All of these decreases were prevented when LPS injection was combined with dexamethasone.

**FUTURE RESEARCH DIRECTION**

The recent cloning of Pept-1 has opened up a fertile ground for the investigation of the molecular and cellular mechanisms of the regulation of intestinal transport of oligopeptides. Indeed, within the past few years, several laboratories have been actively involved in this investigation. The results have identified the products of protein digestion and certain hormones as metabolic signals for modulation of either the transcription of the gene encoding Pept-1 or the intracellular trafficking of Pept-1 protein. However, the pathways for the transmission of these signals remain to be elucidated. In addition, although there have been studies of Pept-1 expression in pathological conditions, much remains to be investigated. For example, is the colonic induction of Pept-1 in inflammatory bowel diseases a primary or secondary event?

As far as clinical application is concerned, the knowledge of regulation of Pept-1 expression has implications in studies of enteral nutrition and drug therapy.
For example, it can be determined whether 1) increasing the expression of Pept-1 increases the efficacy of drug therapy for common clinical conditions such as infection and hypertension, 2) decreasing the expression of Pept-1 decreases the progression of ulcerative colitis and Crohn’s disease, and 3) patients with diabetestes or infectious diarrhea requiring enteral nutrition may have a better protein nutrition with dipeptides rather than amino acids as the nitrogen source.

REFERENCES


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INVITED REVIEW


