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REVIEW ARTICLE

Understanding the role of tryptophan and serotonin metabolism in gastrointestinal function

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Abstract *Tryptophan is the precursor of a wide array of metabolites, which are involved in a variety of aspects of human nutrition and metabolism. Accumulating evidence suggests a role of tryptophan metabolites, especially serotonin (5-hydroxytryptamin) in intestinal (patho) physiology, although mechanisms of action are still poorly understood. Alterations of serotonin metabolism may give rise to gastrointestinal dysfunction. Recently, it has been postulated that other metabolites of tryptophan, mostly of the kynurenine pathway, also play a role in regulating gut function. This review analyses the current knowledge of the interrelationship between tryptophan metabolic pathways and summarizes the existing scientific evidence regarding the role of tryptophan metabolites in intestinal function and in the pathogenesis of gastrointestinal diseases.*

Keywords *gastrointestinal function, kynurenine, nutritional modulation, serotonin, tryptophan.*

Abbreviations: 5-HT, serotonin; LNAA, large neutral amino acid; CNS, central nervous system; ENS, enteric nervous system; TPH, tryptophan hydroxylase; AADC, aromatic amino acid decarboxylase; 5-HTP, 5-hydroxytryptophan; 5-HIAA, 5-hydroxy indoleacetic acid; MAO, monoamino oxydase; IBS, irritable bowel syndrome; TDO, tryptophan 2,3-dioxygenase; IDO, indoleamine 2,3-dioxygenase; IBD, inflammatory bowel disease; IAcrA, indolyl acrylic acid; IAcrGly, indolylacryloyl glycine.

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INTRODUCTION

Serotonin (5-hydroxytryptophan, 5-HT), a metabolite of the essential amino acid tryptophan, has been the subject of intense biological research since the early 1950s. With increasing knowledge, it has emerged as a mediator of several functions in the human body, including mood, appetite and hemodynamics. Over the past 20 years, serotonin has also gained recognition in the regulation of gastrointestinal motility, secretion and sensation and has served as a basis for development of novel treatments for gastrointestinal disorders.[1,2] Recently, the involvement of other tryptophan metabolites in intestinal function has been postulated, which also implicates the clinical relevance of the interrelationship of the different tryptophan metabolic pathways. Of particular importance is the accumulating evidence on the biological role of metabolites of the kynurenine pathway, which are probably involved in the pathogenesis of a number of gastrointestinal disorders. Derangements in this pathway can indirectly lead to changes in serotonin metabolism, which can in turn lead to gastrointestinal dysfunction. We anticipate that the kynurenine pathway will emerge as key modulator in maintaining intestinal homeostasis. Also, metabolic products of human intestinal microbiota will receive increasing attention. By virtue of their catalytic activity, the human gut microbiota have a considerable impact on gastrointestinal function and host health, which is currently subject to intensive investigation. This

review will provide a summary of recent developments and will give an insight into the role of tryptophan metabolism and metabolites in intestinal physiology. In addition, clinical implications and perspectives of strategies modulating tryptophan metabolic pathways will be discussed.

ABSORPTION OF TRYPTOPHAN

L-tryptophan is an essential amino acid, with an estimated dietary requirement of 5 mg kg^{-1} day^{-1}. It is the limiting amino acid in nearly all protein sources which are of importance for human nutrition, accounting for 1–1.5% of total amino acids in typical plant and animal proteins, respectively.[3] Food products containing relatively high tryptophan content are eggs, milk, meat, soybean, potatoes, cereal, broccoli, cauliflower, eggplant, kiwi fruit, plums, bananas, walnuts, fish, seafood and tomatoes.

The intestinal absorption of orally ingested tryptophan on the apical membrane of enterocytes is mediated via the B^0AT1 (Solute Carrier 6A19, SLC6A19) epithelial amino acid transport system, which is also responsible for the absorption of all other neutral amino acids and employs a Na^+ co-transport mechanism. With the exception of lysine, all other neutral amino acids have a higher affinity to the transport system than tryptophan. A defect in this transport system results in Hartnup disorder, first described in 1956. This is an autosomal recessive disease, characterized by renal aminoaciduria, pellagra-like skin rash and episodes of cerebellar ataxia. An almost complete lack of intestinal tryptophan absorption was demonstrated *in vivo* and in biopsy material *in vitro* in patients suffering from this condition, suggesting that the transporter affected in Hartnup disorder is the major mediator of intestinal tryptophan uptake. The pellagra-like skin disorder, which often occurs in Hartnup disorder similar to the classical pellagra caused by dietary niacin deficiency, is the result of the lack of niacin's precursor tryptophan. Impaired absorption leads to the bacterial degradation products indole and derivates, which in turn can play a causative role in cerebellar ataxia. Protein-rich diets usually abolish the symptoms, most likely due to compensation by transport of peptides, although tryptophan breakdown products are still detectable in urine.[4]

The transporter for tryptophan on the basal membrane of enterocytes is the basolateral aromatic amino acid transporter TAT1 (Slc16a10) protein. This transporter protein is thought to be involved in the pathogenesis of the so-called blue diaper syndrome, whereby malabsorption of tryptophan leads to an overproduction of the bacterial degradation product indican, which is oxidized to indigo causing the blue colour of diapers.[5] To which extent absorption of tryptophan is impaired under this condition remains to be established.

The uptake of tryptophan by peripheral cells, such as tissue macrophages, has not fully been identified yet. Much more is known about tryptophan uptake across the blood brain barrier, which plays a critical role in regulating brain serotonin synthesis. This process is based on competitive transport shared by several large neutral amino acids (LNAAs). Therefore, increases in the plasma concentration of LNAAs decrease the rate of tryptophan uptake into the brain and hence brain 5-HT synthesis.[6] Very little is known about the mechanism underlying uptake into neurons and other cells in the central nervous system (CNS).

METABOLISM OF TRYPTOPHAN

Once in the gastrointestinal tract, tryptophan can enter a number of metabolic pathways: protein synthesis, serotonin pathway, kynurenine pathway and bacterial degradation.

Protein synthesis

Given its limited availability in food, tryptophan is often the rate-limiting amino acid in protein synthesis. There is considerable dispute regarding the extent to which dietary tryptophan is incorporated into protein. Some authors suggest a majority of 90%,[3] others 30%,[7] but other sources agree that there is no net new protein synthesis in steady state nitrogen balanced conditions, therefore, the proportion of dietary tryptophan incorporated in protein is minimal.[8]

Serotonin pathway

About 1–2% of dietary tryptophan is converted to serotonin.[8] Serotonin plays an important role in regulating a number of functions in the human body and serves as the precursor for melatonin synthesis in pinealocytes.

Kynurenine pathway

The kynurenine pathway is the most tryptophan-consuming metabolic pathway. About 95% of the ingested tryptophan enters the kynurenine pathway, which can result in the production of NAD, kynuramines, kynurenic acid, quinolinic acid, picolinic acid but most tryptophan is completely metabolized to yield CO_2 and ATP via the glutarate pathway.[3]

Bacterial degradation in the gut lumen

Approximately 4–6% of tryptophan undergoes bacterial degradation yielding indole, indican, and indole acid derivates.[3,8] Approximately 0.5% of ingested tryptophan is excreted unchanged in urine.[8]

The serotonin and kynurenine pathways and the bacterial degradation of tryptophan will be discussed in detail below. Fig. 1 provides a schematic representation of these different metabolic pathways.

SEROTONIN PATHWAY

Synthesis There is quite extensive knowledge on the importance of serotonin in regulating gastrointestinal function.[1,2] It is one of the most important signalling molecules within the gut, where it plays a pivotal role in initiating secretory and motor reflexes. Approximately 95% of the total serotonin content of the human body is present in the gut, of which 90% is stored in the enterochromaffin cells of the intestinal mucosa. The remaining 10% is largely attributed to serotonergic neurons of the enteric nervous system (ENS). These cells possess the apparatus to produce and store serotonin and hence play an important role in regulating serotonin homeostasis.[1,2] Platelets are unable to synthesize serotonin themselves, but possess a high-affinity uptake system and hence accumulate high concentrations of serotonin derived from the GI tract.

Serotonin is synthesized from tryptophan through hydroxylation and decarboxylation. These processes are catalysed by the tryptophan hydroxylase (TPH) and the aromatic acid decarboxylase (AADC), respectively, the former being the rate-limiting step in the synthesis.[1,2] Tryptophan hydroxylase (tryptophan 5-monooxygenase) expression is limited to a few specific cells: neurons, pinealocytes, mast cells, mononuclear leukocytes, intestinal enterochromaffin cells and bronchopulmonary neuroendocrine epithelial cells. There are two known isoforms of TPH. Tryptophan

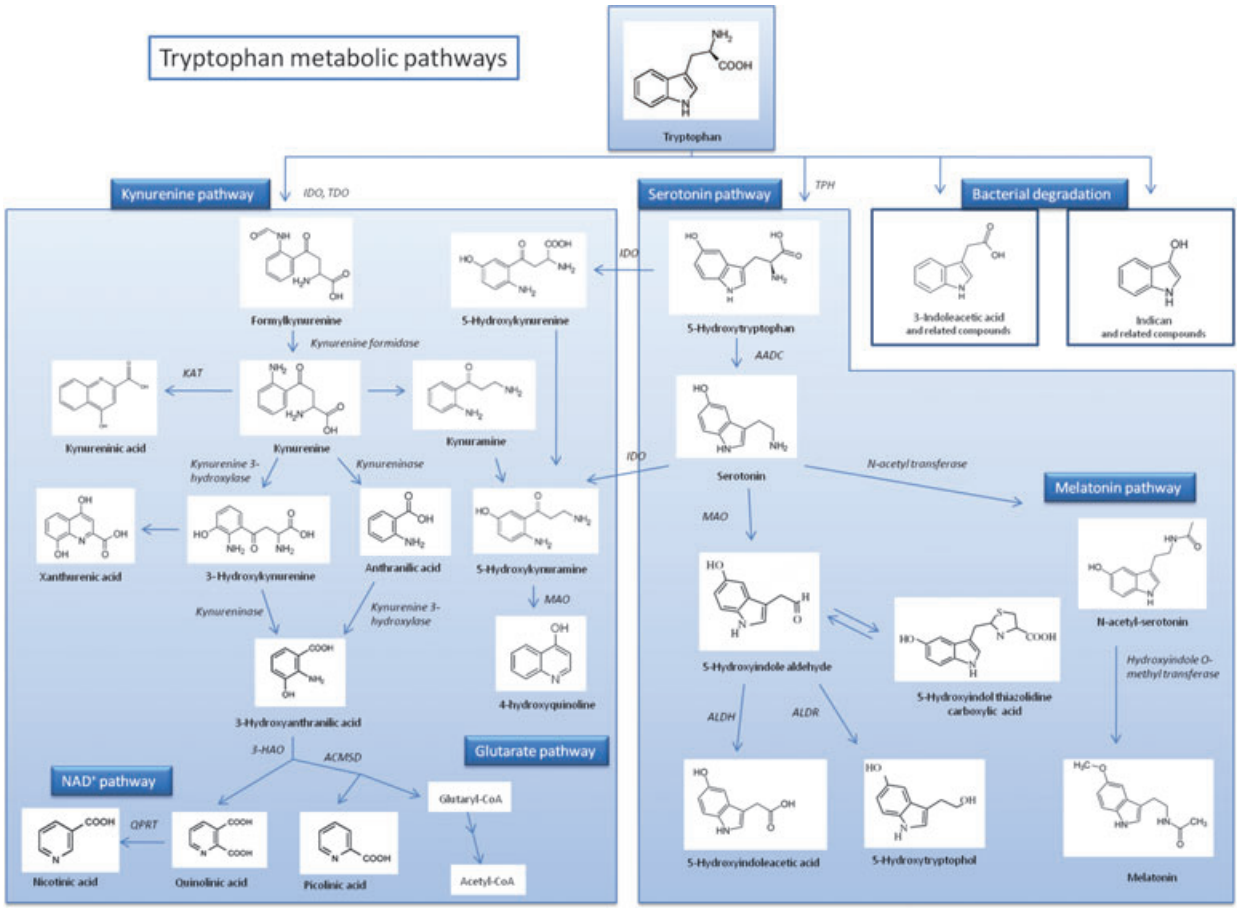

Figure 1 Tryptophan metabolic pathways. TDO, tryptophan 2,3-dioxygenase; IDO, indoleamine 2,3-dioxygenase; KAT, kynurenine aminotransferase; MAO, Monoamine oxydase; 3-HAO, 3-hydroxyanthranilic acid oxydase; ACMSD, aminocarboxymuconate-semialdehyde decarboxylase; QPRT, quinolinic acid phosphoribosyl transferase; TPH, tryptophan hydroxylase; AADC, aromatic amino acid decarboxylase; ALDH, aldehyde dehydrogenase; ALDR, aldehyde reductase.

hydroxylase 1 is localized in enterochromaffin cells and the pineal gland and TPH2 is present in neurons, including serotonergic neurons of the ENS.[9] Both isoforms employ molecular oxygen and the cofactor tetrahydrobiopterin to convert tryptophan to 5-hydroxytryptophan (5-HTP), which is then converted by AADC to serotonin. Aromatic amino acid decarboxylase, which employs vitamin B6 as cofactor, is found in all aminergic cells of the central and peripheral nervous system and is distributed widely in non-neural tissue.[10]

There are at least 14 different neuroendocrine cells of the GI tract, one of them being the serotonin-producing enterochromaffin cells. Serotonin is stored in large dense core vesicles and synaptic-like microvesicles in enterochromaffin cells and is released as result of mechanical or chemical stimulation.[11] Enterochromaffin cells function as sensory transducers. Serotonin released from enterochromaffin cells stimulates mucosal nerve endings of intrinsic primary afferent neurons of the enteric nervous system, which coordinate secretory and motor functions of the intestine.[1,2] Serotonin also activates vagal afferent nerve endings of extrinsic primary afferent neurons projecting to the vomiting centre in the brainstem.[1]

Carcinoids are rare tumours derived from enterochromaffin cells. The carcinoids are most commonly observed in the GI tract and the bronchopulmonary system, which can produce bioactive amines such as serotonin. A subset of patients with carcinoid tumours suffer from carcinoid syndrome, characterized by flushing, diarrhoea, cramps, skin abnormalities, asthmatic wheezing and valvular heart disease associated with elevated 5-HT levels.[12] In carcinoid syndrome patients, an overwhelming majority of tryptophan is converted to serotonin. Some foregut carcinoid tumours are AADC-deficient, and are therefore unable to convert 5-HTP to 5-HT, and may consequently secrete 5-HTP and to lesser extent serotonin.[13] Increased rates of serotonin turnover indicated by elevated urinary 5-hydroxy indoleacetic acid (5-HIAA), a major metabolite of serotonin, can lead to depletion of tryptophan as precursor for other metabolic pathways leading to conditions such as pellagra-like symptoms due to deficient niacin synthesis.[8] A weak inhibitor of TPH, para-chlorophenylalanine (pCPA) has proven effective in the treatment pellagra and diarrhoea in carcinoid syndrome patients and in chemotherapy induced emesis.[14] Unfortunately, administration of pCPA has been associated with the onset of depression and other alterations in CNS function. Recently, a novel class of gastrointestine-selective TPH inhibitors has been discovered, which may provide potential treatment for a variety of gastrointestinal diseases caused by the dysregulation of the serotonergic pathway.[15]

Serotonergic neurons are the minor source of serotonin in the gastrointestinal tract. Small populations of interneurons that can synthesize, take up and release 5-HT have been identified in the human ENS.[1] In guinea pigs, these neurons constitute 2% of the total amount of neurons in the myenteric plexus and their targets project to other submucosal and myenteric ganglia. About half of the myenteric neurons receive serotonergic input and the serotonergic interneurons themselves are targets for 5-HT innervations.[16] Serotonergic interneurons play a role in both motor and secretory reflexes by providing synaptic inputs to motor neurons and ascending interneurons.[17]

Metabolism of serotonin

Serotonin is metabolized by several metabolic pathways. The majority of serotonin is catabolized by monoamine oxidase (MAO). This enzyme is located in mitochondria of a wide range of cells and catalyses oxidative deamination of several biogenic amines. At least two forms of MAO are known (MAO-A and MAO-B). MAO-A has the highest affinity for serotonin.[18]

5-hydroxyindole acetaldehyde (5-HIAL), the product of oxidative deamination of serotonin can be metabolized to 5-HIAA or 5-hydroxytryptophol (5-HTOL). The former reaction is the major pathway under normal conditions. This reaction is catabolyzed by aldehyde dehydrogenase, employing NAD as coenzyme. Reduction of the aldehyde intermediate is catalysed by aldehyde reductase, which employs NADH as coenzyme.[19] In case of a 5-HT overload, the known 5-HT catabolic pathways may become overloaded, allowing lesser-used pathways to convert more 5-HT thereby increasing levels of 5-HT metabolites that might not readily be observed under other conditions.

5-hydroxytryptophol is a minor serotonin metabolite under normal conditions, accounting for 1% of the total serotonin turnover. Alcohol consumption leads to an increased synthesis of 5-HTOL and a concomitant decrease in the synthesis of 5-HIAA, resulting in an increased 5-HTOL/5-HIAA ratio, which is a sensitive marker for detection of recent alcohol intake. During ethanol oxidation the conversion of serotonin shifts away from oxidation of the intermediate 5-HIAL producing 5-HIAA toward the reductive pathway forming 5-HTOL. This has been attributed to the competitive inhibition of aldehyde dehydrogenase by ethanol-derived acetaldehyde.[19]

Another proposed metabolic pathway of serotonin involves glucuronidation. Serotonin has been charac-

terized as a highly selective substrate of human UDP-glucuronosyltransferase UGT1A6 in Caco-2 cells, an *in vitro* model of human intestinal epithelium. These findings suggest that UGT1A6 contributes to the homeostatic control of intestinal 5-HT metabolism.[20] However, studies on the expression of UGT1A6 in human intestinal epithelium are not conclusive. Munzel *et al.* demonstrated the expression of UGT1A6 mRNA in human duodenum with large interindividual differences,[21] while Radominska-Pandya *et al.*[22] found that UGT1A6 was not present in the human intestine, suggesting a regulational control of intestinal 5-HT metabolism that remains to be elucidated.

Recently, the metabolite 5-hydroxyindole thiazolidine carboxylic acid (5-HITCA) has been identified in rodent CNS and ENS tissue samples. This is a condensation product of 5-hydroxyindole acetaldehyde and L-cysteine.[23] 5-HITCA can be detected natively in homogenizated rodent ENS samples. In 5-HT incubated central and enteric nervous system tissue samples, 5-HITCA forms at levels equivalent to 5-HIAA. An equilibrium between 5-HITCA and 5-hydroxyindole acetaldehyde coupled to the enzyme aldehyde dehydrogenase, in the CNS and ENS suggests equilibrium prevents this accumulation.[23]

Imbalances in 5-HT levels within the ENS and the intestine have been associated with various functional gastrointestinal disorders. The most attention perhaps has been given to irritable bowel syndrome, which has been documented extensively.[1,24] Irritable bowel syndrome (IBS), affecting approximately 15–20% of the adult population, is a functional intestinal disorder characterized by abdominal pain or discomfort associated with altered bowel habits, without indications for an organic cause. An increased number of enterochromaffin cells and altered mucosal serotonin metabolism have been described in IBS and serotonergic compounds have been shown to beneficially influence intestinal motor and sensory function.[1] A functional hallmark of IBS is visceral hypersensitivity, which is present in approximately 50% of patients with IBS.[25] Previous research highlights the involvement of the 5-HT_3 receptors on extrinsic primary afferent neurons of the ENS in mediating the pain sensation.[1] It was also suggested previously that the increased visceral sensitivity observed in IBS patients may at least partly be caused by a decreased epithelial integrity, causing intraluminal compounds to cross the epithelial barrier and trigger the ENS and, thereby, nociception.[26] Serotonin could therefore also play a role in regulating the permeability of the intestine.

Tight regulation of 5-HT levels in nervous tissue and intestinal mucosa is necessary and 5-HT catabolism plays an important role in this regulation. Understanding the catabolic pathways, and knowledge on the enzymes involved and the products of these conversions are particularly important because catabolism is vital to regulation of 5-HT levels. Elucidating 5-HT synthetic and catabolic pathways may provide novel approaches for the therapies designed to treat disorders associated with 5-HT homeostasis.

KYNURENINE PATHWAY

In adult young men, about 95% of dietary tryptophan is metabolized along the kynurenine pathway.[3] The biological functions of the kynurenine pathway are: clearance of excess tryptophan and regulation of plasma tryptophan levels, maintenance of nicotinic acid levels, regulation of CNS function and enhancement of macrophage defence function.

Entering the kynurenine pathway, tryptophan is first oxidized by tryptophan 2,3-dioxygenase (TDO), which is almost entirely localized at hepatic cells. Tryptophan 2,3-dioxygenase is the rate limiting enzyme for kynurenine synthesis in the periphery. Tryptophan 2,3-dioxygenase expression and activity can be induced four- to tenfold by tryptophan loading within a period of a few hours.[8] The principal branch of the kynurenine pathway generates quinolinic acid and nicotinamide, whereas the side chains generate kynurenic acid and xanthurenic acid (see Fig. 1). Several biological features of kynurenine metabolites have been described. Most attention has been given to the imbalance in neurotoxic and neuroprotective properties of these compounds, which have been associated with several CNS pathologies. Quinolinic acid is considered to be an excitotoxic N-methyl D-aspartate (NMDA) receptor agonist, whereas kynurenic acid is a neuroprotective NMDA antagonist and an $\alpha 7$ nicotinic cholinergic agonist.[27] In mononuclear cells, including tissue macrophages, quinolinic acid is the main end product of the kynurenine pathway and plays a role in immunoregulatory processes.[28]

The kynurenine pathway also provides the precursors for the dietary supplement niacin, a collective term for nicotinamide and nicotinic acid. Under normal conditions, most of the tryptophan that enters the oxidative pathway is converted to CO_2 and water in the glutarate pathway. Only if this branch of the pathway is saturated, NAD becomes a major product of metabolism.[3] Although metabolites of the glutarate pathway are present in many tissues, including the intestine, NAD synthesis is only possible in the liver, because this is the only organ that possesses all the necessary enzymes.[28]

Another product of the kynurenine pathway is picolinic acid. Picolinic acid is only produced when the flux of metabolites through the glutarate pathway is high and enzymes of the glutarate pathway are saturated.[3] Picolinic acid acts as a chelating agent of elements such as chromium, zinc, manganese, copper, iron, and molybdenum in the human body. It forms a complex with zinc that may facilitate the passage of zinc through the gastrointestinal wall and into the circulatory system.[8] Several of the enzymes of the kynurenine pathway use vitamin B6 as cofactor and a key feature of the enzyme kynureninase is its exceptionally high sensitivity to pyridoxine deficiency. Lack of vitamin B6 leads to a large increase in xanthurenic acid excretion. This has been used for decades as a diagnostic test for vitamin B6 deficiency.[8] Vitamin B6 deficiency also compromises serotonin synthesis, and hence can lead to competition between the two pathways for the co-factor.

Besides TDO, another enzyme initializing the kynurenine pathway is indoleamine 2,3-dioxygenase (IDO). Indoleamine 2,3-dioxygenase is widely distributed in peripheral tissues. The human intestine contains a relatively large amount of IDO.[29] While TDO exclusively accepts tryptophan as substrate, IDO has a broader specificity and can also take 5-HTP, 5-HT and tryptamin.[29] The expression of IDO increases in response to infection and inflammation, with interferon-γ being the strongest stimulator. Mononuclear cells that synthesize IDO reduce extracellular tryptophan concentration so that adjacent T-cells, which depend on tryptophan from the extracellular environment, are unable to activate and proliferate upon encountering antigens. Therefore, IDO might play a role in preventing the initiation of autoimmune disease by enforcing T-cell tolerance through suppressing their proliferation.[28] Hence, high local expression of IDO by mononuclear cells may represent an anti-inflammatory and immunosuppressive mechanism tempting to counterbalance tissue damage.[30] This mechanism could be involved in intestinal pathophysiology, as IDO expression is markedly induced in lesional colonic biopsies of inflammatory bowel disease (IBD) patients[30] and increased IDO activity has been observed in patients with celiac disease[31] and diverticulitis.[32] A similar IDO-based intrinsic immunoescape mechanism is probably employed by colon tumour cells.[33]

Besides through the regulatory effect of IDO on T-cells and immune function, inflammatory responses in the ENS and the gastrointestinal tract related to the kynurenine pathway can also be based on a sensitive balance between the pro-inflammatory, excitotoxic quinolinic acid and the anti-inflammatory, neuroprotective kynurenic acid.[34] This balance could have profound influence on the excitability of enteric neurons, which can affect intestinal motor and sensory function. Increased levels of the kynurenine pathway metabolites kynurenine and kynurenic acid have been observed in sera of patients with inflammatory bowel disease.[35] The increased activity of the kynurenine pathway may represent either a compensatory response to elevated activation of enteric neurons or a primary abnormality which induces a compensatory increase in gut activity. In either case, the data may indicate a role for the kynurenine modulation of glutamate receptors in the symptoms of IBD.[35]

Recent evidence suggests the involvement of the kynurenine pathway in intestinal motility, although the exact roles of kynurenine metabolites in intestinal motor function remains unclear.[34] Kynurenic acid acts as an antagonist on NMDA receptors on enteric glutamatergic neurons and may cause dysregulation of intestinal motility. Glutamate is likely to play an excitatory role and may modulate cholinergic transmission in the ENS.[36] Glutamate immunoreactivity has been detected in submucosal and myenteric neurons in the guinea pig ileum, and NMDA receptors are present on enteric cholinergic neurons, and vagal and spinal primary afferent nerve endings. N-methyl D-aspartate receptor activation has been shown to stimulate acetylcholine release from myenteric neurons, thereby modulating smooth muscle contraction.[36] Recent result have revealed a significant potential for kynurenic acid to decrease the facilitary pathways of colonic motility.[34] Kynurenic acid might also play a role in intestinal mechanosensitivity, as it has been proven to act also as glutamate antagonist and inhibit mechanosensitivity of both mucosal and tension vagal afferents.[37] Furthermore, kynurenic acid also exerts an anti-inflammatory effect due to inhibition of xanthine oxydase, resulting in less reactive oxygen species (ROS) production.[34]

Other products of the IDO and formamidases are kynuramin derivates. Formation of kynuramines has been described in various tissues, including the intestine and appears to be directly proportional to tryptophan concentrations. Kynuramines may be important as endogenous agonists or antagonists of 5-HT receptors in smooth muscle. Marked non-selective serotonergic agonist properties of 5-hydroxykynuramine at multiple 5-HT receptors were demonstrated in rat ileum. 5-hydroxykynuramine is formed from tryptophan to much lesser extent *in vivo* than 5-HT, but pathological conditions or situations in which tryptophan concentrations are increased may lead to an

overproduction of kynuramines. Besides its effect on smooth muscle, 5-hydroxykynureamine is a potent inhibitor of the action of serotonin in promoting the aggregation of platelets. This may provide a measure of regulation in cases of over-synthesis of serotonin, not only as an alternative catabolic pathway of the amine, but also to inhibit one of its biological actions.[38]

BACTERIAL DEGRADATION PRODUCTS

Indican The main bacterial breakdown product of tryptophan is indole. The wide range of bacterial species capable of producing indole include *E. coli*, *Proteus vulgaris*, *Paracolobactrum coliforme*, *Achromobacter liquefaciens* and *Bacteriodes* spp. The formation of indole is catabolyzed by the enzyme tryptophanase, which is inducible by tryptophan and repressible by glucose in most bacteria.[39] By-products of this conversion are pyruvate, which can be used in fermentation or respiration reactions, and ammonia, which can have potentially toxic effects on the intestinal epithelium. High protein diets are therefore able to induce bacterial tryptophanase activity, which in turn results in overproduction of indole and other compounds that can thereby reach toxic concentrations in the colon.[39]

After absorption, indole is oxidized to indoxyl, conjugated with sulphate and excreted as urinary indican (also known as indoxyl-sulphate). In normal individuals only small proportions of dietary tryptophan reach the colon because of nearly complete absorption in the small intestine. Approximately 3% of dietary tryptophan is excreted as urinary indican.[8] Because the upper gastrointestinal tract is sparsely populated with bacteria, indican is present in urine at low levels in healthy individuals. An elevated level of urinary indican can be an indication of upper bowel bacterial overgrowth. Nevertheless, even patients with normal intestinal bacterial populations can show increased postprandial indican excretion when they fail to digest dietary protein. Although not used in everyday medical practice due to availability of more specific tests, the relationship between increased indican and incomplete digestion may serve as measure of protein digestive adequacy (Obermeyer test).[8]

Indican is also known to be a nephrotoxin that accumulates in the blood of patients suffering from chronic kidney failure. Because tryptophanase activity derives from only a subset of enteric bacteria, non-indole-producing bacteria, such as various *Bifidobacterium* species, have been administered as a test probiotic to dialysis patients to decrease their plasma levels of indoxyl sulphate.[40] Excessive indican excretion also occurs in a rare condition known as the purple urine bag syndrome, first described in 1987. It is associated with urinary tract infections occurring in catheterized patients, generally elderly females with significant co-morbidities and constipation, in which case excess indican is transformed to indirubin and indigo in alkaline urine in the presence of sulphatase/phosphatase producing Gram negative bacteria.[41]

Indolic acid derivates A smaller quantity of tryptophan is converted by bacterial action to indolic acid derivates: indolyl-3-acetic acid, indolyl-acetyl-glutamine, indolyl-propionic acid, indolyl-lactic acid, indolyl-acrylic acid and indolyl-acryloyl-glycine. Intestinal microorganisms, including *Bacteriodes*, *Clostridia* and *E. coli*, catalyse tryptophan to tryptamin and indolyl-pyruvic acid, which are then converted to indolyl-3-acetic acid, indolyl propionic acid and indole lactic acid.[39] Indolyl acetic acid can be conjugated with glutamine in the liver to yield indolylacetyl glutamine. Indolylpropionic acid can be further converted in the liver or kidney into indolyl acrylic acid (IAcrA) and conjugated with glycine to produce indolylacryloyl glycine (IAcrGly). Some evidence suggests that IAcrA can also be produced in the absence of intestinal microorganisms, although there is no direct evidence for enzymatic or non-enzymatic processes.[42] Nutritional intervention such as tryptophan loading did not influence urinary IAcrGly levels, but complete elimination of tryptophan from the diet resulted in a marked decrease of IAcrGly in urine, similarly to parenteral alimentation.[42]

The biological role of these compounds still needs to be investigated. Increased and prolonged excretion of urinary indols (e.g. indican, indolyl-3-acetic acid, indolyl-3-acetyl-glutamine, indolyl-lactic acid, indolyl-acryloyl-glycine) has been observed in a number of diseased states including Hartnup disorder, celiac disease and other malabsorptive states.[43] It is assumed that this is due to excessive tryptophan overload in the colon possibly with coexistent alteration in gut microbiota, which leads to increased production of bacterial degradation products.[43]

Some bacterial products are toxic to other microbiota, and this provides competitive advantage for the producers. Some indolic compounds are known to have bacteriostatic effect on Gram negative enterobacteria, especially within the genera *Salmonella* and *Shigella*.[39] The increased urinary excretion of indolic compounds reflects variations in gut microbiota composition in relation to nutritional competition. For instance, indolyl acetic acid has been reported to inhibit the growth and survival of *Lactobacilli*, and specifically *L. paracasei*.[44]

Also, indolyl propionic acid has been shown to be a powerful antioxidant, and is currently being investigated as a possible treatment for Alzheimer's disease.[40]

Some authors suggest that indolylacryloyl glycine could be a possible marker for autism and is also associated with increased intestinal epithelial permeability.[45] Autism has been associated with increased intestinal permeability.[46] Researchers from Sunderland, UK, hypothesized that the changes in intestinal permeability are a result of membrane damage through the precursor of IAcrGly, indolyl acrylic acid, a planar and very reactive molecule. Elevated levels of IAcrA are detectable in urine by measuring its metabolite IAcrGly. The membrane dysfunction results in an increased permeability and permeation of compounds which could influence normal homeostasis, including CNS development, which may result in pervasive developmental disorders or autism.[45] This group has also suggested a gluten-free diet which decreases the urinary levels of IAcrGly and ameliorates some of the symptoms of the pervasive developmental disorder. Their theory is very plausible, but not generally accepted.[47]

COMMON GASTROINTESTINAL DISORDERS

Recent studies suggest the involvement of tryptophan metabolic routes in the pathogenesis of several common gastrointestinal disorders, such as IBD, IBS, celiac disease and diverticulitis.

A common mechanism could possibly be the upregulation of IDO. Elevated kynurenine and kynurenine/tryptophan ratios, indicative of increased IDO activity, have been observed under inflammatory conditions, such as IBD, which result in altered T-cell proliferation and survival.[30] Such a mechanism could also account for altered tryptophan catabolism in IBS patients, as studies have reported immune activation and pro-inflammatory cytokine production in IBS.[48]

Moreover, upregulation of IDO induces a metabolic shift, which is presumably also involved in the pathogenesis of IBS. Recent studies have shown that both females[49] and males[50] with IBS have increased kynurenine concentrations compared to controls. Furthermore, a positive correlation was found between IBS severity and the kynurenine/tryptophan ratio. Those with severe IBS symptoms have increased shunting of tryptophan along the kynurenine pathway which contributes to the abnormal serotonergic function.[49] Altered serotonergic conditions have directly been associated with malfunction of the intestine in IBS.

Besides a role in the pathological states of the intestines, serotonin has also been suggested to be involved in the pathogenesis of non-alcoholic steatohepatitis. Degradation of serotonin by MAO also yields ROS as byproducts. Increased uptake and catabolism of serotonin in the liver therefore leads to overproduction of ROS resulting in hepatocellular injury by mitochondrial damage and inflammation.[2] In recent years, the understanding of the (patho)physiological role of tryptophan metabolites, mostly serotonin, in the gastrointestinal tract has increased significantly.[1,2,31,34,35,49] Nevertheless, further investigation will be needed to assess the biological role of other tryptophan derivates. This will lead to a better comprehension of the pathogenesis of the most common causes of gastrointestinal morbidity.

NUTRITIONAL MODULATION OF TRYPTOPHAN METABOLISM

The question arises to which extent tryptophan and serotonin metabolism can be influenced by nutrition and how this affects gut function. In-depth understanding of pathways involved in tryptophan and serotonin metabolism is therefore crucial. Furthermore, the interrelationship between metabolic pathways is of profound pharmacological and physiological importance, in that changes in one pathway might have secondary effect on the others.

Nutritional intervention has been employed since decades in investigating the serotonergic system, which has been proven to be involved in the pathogenesis of several psychiatric disorders, mainly depression. It is also well known that depression and other psychiatric disorders often coincide with functional gastrointestinal disorders and that serotonin is a mediator between brain and intestine, the so-called brain-gut axis.[51] Furthermore, serotonin also has a pivotal role in regulating appetite, satiety and food intake.[52]

Experimental evidence in humans suggests that an acute decrease in 5-HT synthesis can be achieved by means of the acute tryptophan depletion (ATD) method. Acute tryptophan depletion employs ingestion of an amino acid mixture devoid of the precursor of 5-HT and subsequently lowers 5-HT synthesis between 4 and 7 h after administration, based on a competitive uptake between tryptophan and other large neutral amino acids. Acute tryptophan depletion has been used over the past decade in the psychiatric setting; recent evidence suggests that it affects the brain-gut axis and intestinal 5-HT metabolism as well. Using the ATD method, alteration of gastrointestinal and anxiety symptoms,[53] and changes in visceral

perception and cognition have been observed in IBS patients.[54]

Historically, tryptophan has been used as an anti-depressive and sleep-inducing substance through increasing 5-HT synthesis.[55] Similar effects have been observed in case of supplementation with a tryptophan-rich protein, alpha-lactalbumine[56] and nutritionally-sourced tryptophan.[57] Furthermore, tryptophan loading increases the catabolism along the kynurenine pathway and can lead to substrate depletion from the serotonin pathway. A similar metabolic shift and subsequent shortage of 5-HT has also been postulated to be responsible for major depression.[58] Inflammatory conditions also lead to induction of metabolism along the kynurenine pathway. Therefore, the direct precursor of serotonin, 5-hydroxytryptophan, has also been used to influence the serotonergic system. A natural source of 5-HTP is the seeds of the West-African plant *Griffonia simplicifolia*. Following oral intake, it is converted by the intestine and liver to serotonin by the action of AADC.[10] Therefore, it can be expected that administration of 5-HTP causes an increase in the intestinal 5-HT levels. A small amount of 5-HTP is taken up by the central nervous system, the reason for its use as an anti-depressant.[55]

Evidence also suggests that the kynurenine pathway itself can be influenced by nutrition.[28] Kynurenic acid has been proposed to have a positive effect in conditions accompanied with intestinal hypermotility.[34] It is present in certain sources of food, such as honeybee products.[59] Therefore, kynurenic acid and its derivates may be used as a therapeutic target for the treatment of functional gastrointestinal disorders,[34] possibly through nutritional intervention.

Imbalance between neuroprotective and neurotoxic activities of the kynurenine pathway have been associated with various CNS pathologies.[27] Based on recent scientific evidence as discussed above, an imbalance of kynurenine pathway metabolites also need to be considered in the development of gastrointestinal dysfunction and intestinal immune function. The biological effects of bacterial degradation products of tryptophan need to be further clarified as well. An overload of dietary proteins or changes in intestinal microbiota can lead to overproduction of these products and subsequently lead to changes in intestinal physiology with potentially toxic consequences. Interest in the impact of gut microbial activity on human health is expanding rapidly, and a better identification of the host-microbiota interactions can also lead to developing new approaches in disease treatment. Probiotic supplementation, for instance, can aim to replace or reduce the number of potentially harmful proteolytic *E. coli* and *Clostridia* producing toxic tryptophan breakdown products by enriching populations of gut microbiota that have more advantageous metabolic activity. Future activities will be directed to influence the gut microbiota in a targeted way, ideally by enhancing beneficial effects and minimizing adverse effects. However, before we are able to do so, further work is required to understand in more detail the processes underlying the bacterial conversion of tryptophan and other dietary components. Given our current knowledge of tryptophan metabolism in the human body, we may assume that nutritional modulation can offer a tool to better understand gastrointestinal functioning and also to open new therapeutic horizons in the treatment of functional gastrointestinal disorders.

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