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Review

Potential role of milk bioactive peptides on the serotonergic system and the gut-brain axis

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ABSTRACT

Dairy-derived bioactive components have health-promoting effects due to their large number of biological properties. Although the physiological significance of several of these substances is not yet fully understood, both proteins and bioactive peptides are now believed to be health-enhancing components. Serotonin is a key signalling neurotransmitter for the gut–brain axis that controls a wide range of physiological functions, highlighting its regulation of neurobiological and intestinal physiology. Here, we discuss the roles and pathways whereby these milk-derived bioactive peptides could modulate serotonergic functions as they are an emerging and potential therapeutic adjuvant for the dietary modulation of gut–brain axis disorders mediated by serotonin.

Contents

1. Introduction

Bovine milk is the most consumed type of milk (Liu, Zhang, Han, Zhang, & Zhou, 2020), and several dairy products have also been consumed worldwide for millennia. The consumption vary considerably among regions and has decreased in Western societies during the last few decades due to the negative health effects attributed to milk and dairy products (Haug, Høstmark, & Harstad, 2007). These harmful health effects are probably due to the high fraction of saturated fatty acids (SFAs) that milk fat contains, which increase low-density lipoprotein (LDL) cholesterol levels, a risk

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factor for the development of cardiovascular diseases (Lordan, Tsoupras, Mitra, & Zabetakis, 2018). However, current evidence indicates that milk and dairy products may reduce the risk of developing cardiometabolic diseases (Fontecha, Calvo, Juarez, Gil, & Martínez-Vizcaino, 2019), obesity (Thorning Tanja et al., 2016), and type 2 diabetes (Hidayat, Du, & Shi, 2019), and they also may protect against specific types of cancer such as colorectal cancer (Lampe, 2011). Many beneficial properties of milk are due to its content in nutritional and functional components with physiological benefits, such as proteins, lipids, carbohydrates, peptides, enzymes, vitamins, and minerals. These compounds are obtained on an industrial scale and considered for application as ingredients in both functional foods and nutraceutical products. Nonetheless, some industrial processing operations used routinely, such as homogenisation and heating, have negative effects on milk components such as protein denaturation or coagulation (Mudgil & Barak, 2019).

Over the last few years the manufacture of bioactive milk ingredients has emerged as an interesting and productive sector for dairy industries and bio-industries (Park & Nam, 2015). Proteins are particularly interesting for their nutritional properties and physiological functions due to their bioactive peptides. Therefore, bovine milk proteins are the most studied for the generation of bioactive food protein hydrolysates (Nongonierma & FitzGerald, 2018). The aim of this review is to assess the biological functions of bovine milk bioactive proteins and peptides and their effects on neurobiological and intestinal physiology through the regulation of the serotonergic system.

2. Milk proteins and bioactive peptides

The protein content of bovine milk is 3.2%, of which 80% are caseins (α_{S1}-, α_{S2}-, β-, γ-, and κ-casein) and the rest are whey proteins (Kekkonen & Peuhkuri, 2009). Bovine whey contains lactose and non-casein proteins of milk that are mainly β-lactoglobulin (β-LG, 50−60%), α-lactalbumin (α-LA, 15−25%), immunoglobulins (Igs, 10%), bovine serum albumin (BSA, 6%), and lactoferrin (LF, < 3%) (Corrochano et al., 2018). However, whey is also made up of other proteinaceous components, such as lactoperoxidase, proteose peptones, and glycomacropeptide, which are released from κ-casein in the first step of enzymatic cheesemaking (Madureira, Tavares, Gomes, Pintado, & Malcata, 2010). Casein is the major component of cheese, and whey is obtained after coagulation and precipitation of casein in cheesemaking. Whey has traditionally been considered a dairy by-product with polluting capacity due to its high organic loading and elevated generated volume. Nevertheless, since it constitutes 20% of milk proteins and retains approximately 55% of milk nutrients, its possible use in the development of functional foods is interesting (Brandelli, Daroit, & Corrêa, 2015; Hernández-Rojas & Vélez-Ruíz, 2014).

Milk proteins represent a source of biologically active peptides associated with health benefits and released by enzymatic hydrolysis (Kekkonen & Peuhkuri, 2009). Some peptides are absorbed from the intestine to the blood and travel to diverse organs and the brain, where some of them can cross the blood-brain barrier barrier (BBB). On the contrary, some peptides act through receptors and cell signalling in the gut without absorption. Bovine milk peptides predominate over those derived from the milk of other species. However, since there is homology in the peptide sequences between species, the studies in bovine milk could be extrapolated to the bioactive peptides of other species (Nielsen, Beverly, Qu, & Dallas, 2017).

2.1. Physiological effects of milk bioactive peptides and proteins

The processing of milk proteins is important to yield protein hydrolysates and bioactive peptides that can trigger different physiological effects in the human body (Zhao & Ashaolu, 2020). Most bioactive peptides are derived from α-, β-, and κ-casein: caseinophosphopeptides, casokinins, casomorphins, isracidin, casocidin, casoxins, casoplatelins, glycomacropeptides and other small peptides. There are also relevant peptides derived from whey such as α- and β-lactorphins, lactokinins, lactostatin, β-lactotensin, serorphin and albutensin A. However, the majority of known whey-derived bioactive peptides are derived from lactoferrin (Nielsen et al., 2017), because this is the most valuable protein present in whey (Krissansen, 2007). Some of these peptides are lactoferricin B, lactoferrampin, and lactoferroxin (Table 1).

Casein, whey, and their peptides, play an important role in the immune system via their antimicrobial, anti-inflammatory, immunomodulatory (Nielsen et al., 2017), and cytomodulatory effects (Mohanty, Mohapatra, Misra, & Sahu, 2016). The antimicrobial action is commonly due to the interaction of positively charged hydrophilic regions with the cell membrane and its subsequent increase in permeability. In turn, the anti-inflammatory activity is mainly based on the inhibition of the nuclear factor-κB (NF-κB) in macrophages, which blocks the transcription of proinflammatory cytokines. In addition, anti-inflammatory peptides also reduce the adhesion of monocytes to circulatory endothelial cells.

Finally, immunomodulatory function includes the regulation of proliferation, differentiation, and activation of immune cells (Rutherfurd-Markwick, 2012). Milk-derived bioactive peptides and proteins also act on the cardiovascular system via their antihypertensive, antithrombotic, and hypocholesterolaemic activities (Nielsen et al., 2017).

On the nervous system, they exert effect through their opioid agonist, antagonist, and ileum-contracting activities (Fernández-Tomé et al., 2016; Kekkonen & Peuhkuri, 2009). The effects elicited by agonist opioids can be diverse, including prolonging GI transit time, inhibiting intestinal peristalsis, modulating amino acid transport and behaviour, drawing out analgesia, inducing respiratory depression, and increasing appetite by stimulating the secretion of insulin and somatostatin (Fernández-Tomé et al., 2016; Rutherfurd-Markwick, 2012). Conversely, antagonist opioids suppress the agonist activity of analgesic peptides, such as encephalin, and lead to appetite reduction, so this peptide could be involved in the prevention and/or treatment of obesity (Nielsen et al., 2017; Rutherfurd-Markwick, 2012).

Some of these dairy derivatives also influence bone physiology, have antioxidant, anticarcinogenic, and weight-management functions (Fernández-Tomé et al., 2016; Kekkonen & Peuhkuri, 2009). Milk is a source of calcium, and some of its proteins, such as caseins, are mineral binding and increase the absorption of calcium in the intestine, which explains its osteoprotective effect (Rutherfurd-Markwick, 2012). Whey proteins promote bone formation and suppress bone resorption (Krissansen, 2007). Moreover, some peptides can eliminate and prevent the formation of free radicals (Nielsen et al., 2017). The antioxidant activity of whey is probably due to the contribution of cysteine-rich proteins that are involved in the synthesis of glutathione, an intracellular antioxidant molecule (Abrahão, 2012). Whey can also protect against cellular oxidation boosting intracellular antioxidant enzymes such as catalase, superoxide dismutase, and glutathione peroxidase (Corrochano et al., 2018).

Table 1
Main bioactive peptides from bovine milk proteins and their main biological functions.

Bioactive peptide	Protein source	Biological function
Caseinophosphopeptides	α_{S1}-, α_{S2}-, β-casein	Cytomodulatory, anticarcinogenic (Park & Nam, 2015) and mineral binding (Rutherfurd-Markwick, 2012)
Casokinins	α_{S1}-, α_{S2}-, β-casein	ACE inhibitory (Silva & Malcata, 2005)
Casomorphins	α_{S1}-, α_{S2}-, β-casein	Opioid agonist (Boutrou, Henry, & Sanchez-Rivera, 2015), immunomodulatory, and anticarcinogenic (Park & Nam, 2015; Pepe, Tenore, Mastrocinque, Stusio, & Campiglia, 2013)
Other small peptides	α_{S1}-, α_{S2}-casein	Antioxidant (Brandelli et al., 2015)
Isracidin	α_{S1}-casein	Immunomodulatory (Phelan, Aherne, FitzGerald, & O'Brien, 2009) and antimicrobial (Park & Nam, 2015)
Casocidin	α_{S2}-casein	Antimicrobial (Zucht, Raida, Adermann, Mägert, & Forssmann, 1995)
Casoxins	κ-casein	Opioid antagonist (Nielsen, Beverly, Qu, & Dallas, 2017)
Casoplatelins	κ-casein	Antithrombotic (Silva & Malcata, 2005)
Glycomacropeptides	κ-casein	Immunomodulatory, antimicrobial (Boutrou et al., 2015), antithrombotic (Park & Nam, 2015), and toxin binding (Phelan et al., 2009)
α-, β-Lactorphins	α-lactalbumin, β-lactoglobulin	Opioid agonist (Rutherfurd-Markwick, 2012), ACE inhibitory (Krissansen, 2007), and ileum-contracting (Madureira et al., 2010)
Lactokinins	α-lactalbumin, β-lactoglobulin	ACE inhibitory (Marcone, Belton, & Fitzgerald, 2017)
Lactostatin	β-lactoglobulin	Hypocholesterolaemic (Rutherfurd-Markwick, 2012)
β-Lactotensin	β-lactoglobulin	Hypocholesterolaemic (Marcone et al., 2017), ACE inhibitory, opioid-neurotensin agonist (Krissansen, 2007; Madureira et al., 2010), and memory consolidation (Ohinata et al., 2007)
Serorphin	Bovine serum albumin	Opioid agonist (Sreeja, Jana, Aparnathi, & Prajapati, 2013)
Albutensin A	Bovine serum albumin	Opioid agonist (Sreeja et al., 2013), ACE inhibitory (Krissansen W., 2007), and C3a and C5a complement receptors agonist (Ohinata et al., 2002)
Lactoferroxin	Lactoferrin	Opioid antagonist (Teschemacher, Koch, & Brantl, 1997)
Lactoferricin B	Lactoferrin	Immunomodulatory, anticarcinogenic, antimicrobial, antioxidant (Van Der Kraan et al., 2004; Wakabayashi et al., 1999), and antithrombotic (Wakabayashi, Takase, & Tomita, 2003)
Lactoferrampin	Lactoferrin	Antimicrobial (Bruni et al., 2016)

Otherwise, some proteins such as lactoferrin and α-lactalbumin reduce oxidative stress due to their iron-chelating properties (Abrahão, 2012; Ha & Zemel, 2003). Additionally, it is believed that whey proteins can improve body weight because low-calcium diets induce calcitropic hormones to promote adipose tissue lipid storage, but this effect can be inhibited with high-calcium diets. These proteins also participate in body protein synthesis because of their high leucine and other important amino acid contents, which are important in the protein synthesis pathway (Ha & Zemel, 2003).

3. Serotonergic system

Serotonin, or 5-hydroxytryptamine (5-HT), derived from tryptophan (Trp), is a key neurotransmitter modulating a wide range of neurobiological processes within the central nervous system (CNS), such as control of sleep, cognitive functions, mood, behaviour, nausea, or maturation of neuronal and glial cells (Nongonierma & Fitzgerald, 2015; Taheri-Kafrani et al., 2011). However, only 5% of the total 5-HT is produced by serotonergic neurons in the CNS via the enzyme tryptophan hydroxylase 2 (TPH2) (Gallegos-Perez et al., 2014). The other 95% is produced in the intestine, where it modulates the whole gastrointestinal tract by regulating intestinal motility, sensation of pain, absorption of nutrients and intestinal secretion of ions and water. 5-HT is mainly secreted by enterochromaffin (EC) cells using the rate-limiting enzyme tryptophan hydroxylase 1 (TPH1) (Sikander, Rana, & Prasad, 2009). The released 5-HT triggers different responses through the activation of a diverse family of 5-HT receptors (5-HTRs) that are distributed throughout the body and classified into 7 families involving at least 15 subgroups (Latorre, Mesonero, & Harries, 2019). 5-HT activity also depends on its extracellular availability, mainly regulated by the serotonin transporter (SERT) located on neuronal and non-neuronal cells, including adrenal chromaffin cells, intestinal epithelial cells, platelets, monocytes, and endothelial cells (Banskota, Ghia, & Khan, 2019). Its extracellular levels are subsequently controlled by the equilibrium between 5-HT synthesis and uptake, which is vital for the triggering of 5-HT effects. Thus, the studies of Trp, serotonin, and its receptors continue to be of great importance, due to its various physiological implications in various organs and systems (Park et al., 2021). However, as an essential amino acid, Trp cannot be synthesised by the organism; therefore, it has to be provided through the diet, and is found mainly within a wide range of dietary proteins. Then, a potential link is made between diet and brain/intestinal functions involving 5-HT.

3.1. Serotonergic system and the gut–brain axis

The gut–brain axis is a bidirectional communication system between the CNS and the enteric nervous system (ENS). Since serotonin is a key signalling molecule at both terminals of this axis, alterations in the 5-HT system are evident in disorders that present dysfunctional communication between the gut and the brain (O'Mahony, Clarke, Borre, Dinan, & Cryan, 2015). This complex communication ensures proper maintenance and coordination of GI functions and permits feedback from the gut to exert effects on mood, motivated behaviour, and higher cognitive functions (Foster, Rinaman, & Cryan, 2017). Brain-gut interaction is carried out by a network of pathways, which encompass: CNS, ENS, autonomic nervous system (ANS), neuroendocrine and neuroimmune pathways, and most importantly, the intestinal microbiota (Kennedy, Cryan, Dinan, & Clarke, 2017). Accumulating evidence points to the critical role of the gut microbiota in regulating the normal functioning of this axis. Particularly, the microbial influence on Trp metabolism and the serotonergic system could be a key point in such regulation. There is also an overlap between processes influenced by the gut microbiota and those that rely on intact serotonergic signalling.

Enteric microbiota have an important impact on the gut–brain axis, interacting locally with intestinal cells and the ENS and with the CNS through neuroendocrine and metabolic pathways (Carabotti, Scirocco, Maselli, & Severi, 2015) (Fig. 1). Microbiota–gut–brain signalling occurs through a number of interrelated mechanisms, including microbial metabolites, by modulating circulating Trp availability, as well as neuro-immune pathways that include the regulation of immune activity and the production of pro- and anti-inflammatory cytokines (Dinan, Stilling, Stanton, & Cryan, 2015;

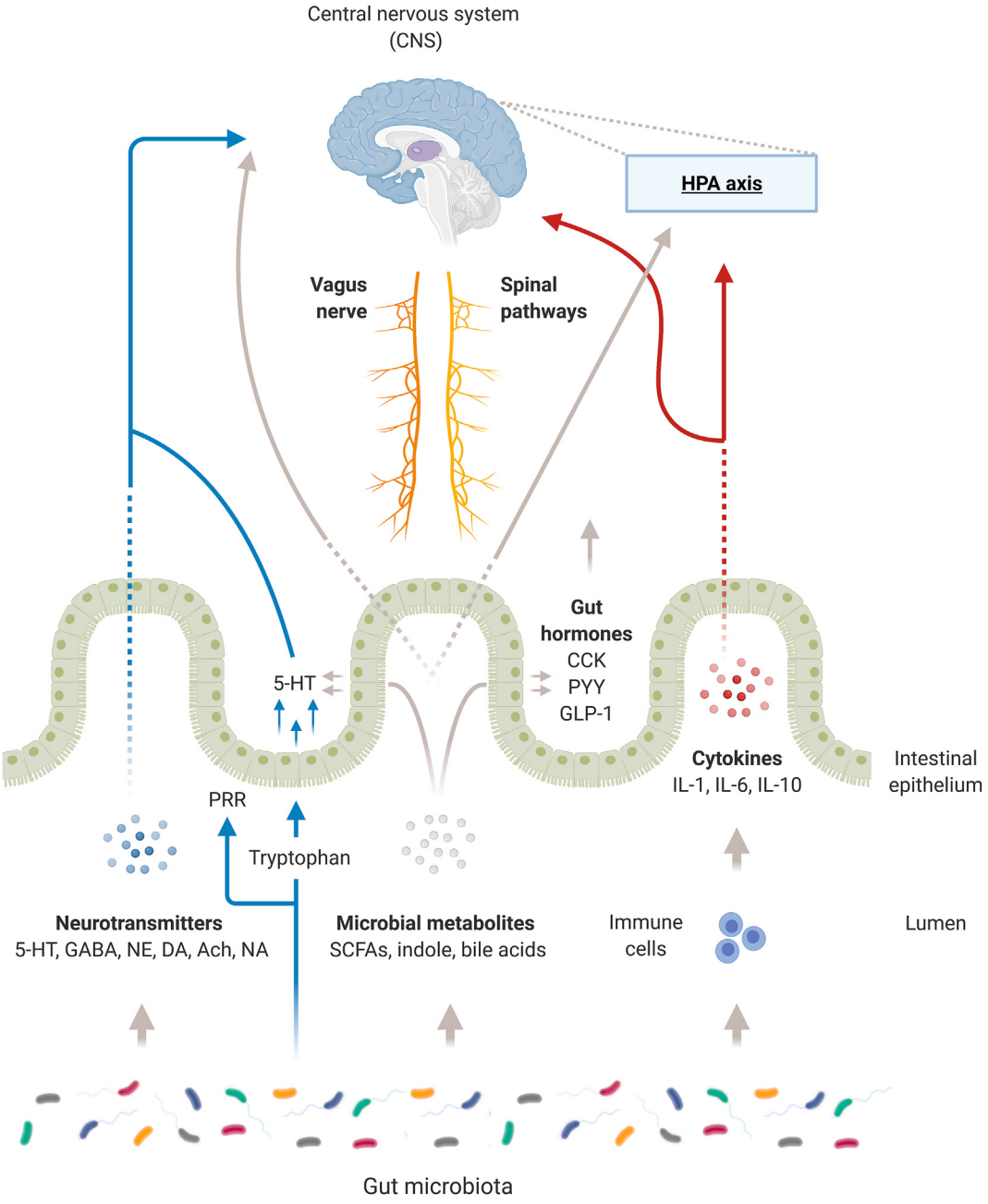

Fig. 1. Communication pathways of the microbiota–gut–brain axis. Microbiota–gut–brain signalling occurs via a network of pathways including: microbial metabolites and neurotransmitters as 5-HT; neuro-immune pathways like proinflammatory and anti-inflammatory cytokines through immune cells, following the stimulation of gut bacteria; the modulation of tryptophan (Trp) metabolism and downstream metabolites. The most important neuronal pathway in mediating the effect of gut microbiota on brain function is the vagus nerve. Neurotransmitters, SCFAs, and cytokines may enter circulation and cross the blood–brain barrier (BBB). Microbial metabolites play an important role in the gut–brain axis because some of them can promote 5-HT synthesis through enterochromaffin cells. 5-HT can cause changes in intestinal serotonergic signalling; however, it is unable to cross the BBB, unlike its chief metabolite, 5-hydroxyindoleacetic acid, which is absorbed from the intestine and passes the BBB. SCFAs can also interact with gut enteroendocrine cells and catalyse the release of gut hormones, which enter circulation and can migrate into the central nervous system (CNS). Likewise, the gut microbiota has an important effect on hypothalamic–pituitary–adrenal (HPA) axis function, since both SCFAs and some cytokines can stimulate the HPA axis to produce corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and cortisol. On the other hand, the interaction of bacteria with certain pattern recognition receptors (PRRs) leads to a decrease in SERT activity, with the consequent intestinal increase of 5-HT, which would lead to alterations in intestinal physiology. Through these routes of communication, the microbiota–gut–brain axis controls gastrointestinal and central processes. Dysregulation of the gut microbiota subsequently leads to alterations in central and peripheral 5-HT signalling, which contributes to stress-related disorders, including anxiety and depression and many chronic gastrointestinal diseases, such as inflammatory bowel diseases (IBD).

Lavelle & Sokol, 2020). Moreover, some bacterial by-products such as short-chain fatty acids (SCFAs) interact with gut mucosal enteroendocrine cells and catalyse the release of gut hormones such as CCK, peptide tyrosine–tyrosine (Peptide YY; PYY), and GLP-1 (Silva, Bernardi, & Frozza, 2020). Both SCFAs and gut hormones enter circulation and can migrate into the CNS (Foster et al., 2017). Gut microbes can communicate with the ENS and the innate immune system through the interaction with pattern recognition receptors (PRRs) along the lumen. The activation of some PRRs, such as NOD2, TLR2, and TLR4, decreases SERT activity and expression, which increases intestinal 5-HT extracellular levels, contributing to the inflammatory response, and affecting the intestinal physiology

(Layunta, Buey, Mesonero, & Latorre, 2021). Such dysregulation of the intestinal serotonergic system leads to symptoms of gastrointestinal disorders (diarrhoea, increased motility, and abdominal pain) and has been related to many chronic GI diseases, including inflammatory bowel diseases (IBD) (Latorre et al., 2016). Beneficial bacteria also precipitate the secretion of anti-inflammatory cytokines such as interleukin-10 (IL-10) (Sarkar et al., 2016), that prevent the development of a chronic inflammatory state and lead to maladaptive changes in mood and behaviour, including increased incidence of stress-related disorders (Foster et al., 2017). In this sense, IL-10 increases SERT activity and expression and restores altered redox equilibrium in intestinal epithelial cells, contributing to reduced inflammatory response in the GI tract (Latorre et al., 2013; Latorre, Matheus, Layunta, Alcalde, & Mesonero, 2014). Other cytokines such as IL-1 and IL-6 provide a potent release of the corticotropin-releasing hormone (CRH) by the hypothalamus, which stimulates the hypothalamic–pituitary–adrenal axis (HPA axis) (Dinan et al., 2015). Bacteria also have the capacity to generate neurotransmitters and neuromodulators such as 5-HT, gamma-aminobutyric acid, norepinephrine or acetylcholine (Gros, Gros, Mesonero, & Latorre, 2021; Lyte, 2011). Neuronal and spinal pathways are critical in mediating the effect of the gut microbiota on brain function and behaviour, being the vagus nerve the most important neural pathway (Kennedy et al., 2017). The gut microbiota and some bacterial metabolites such as long-chain fatty acids have also an important effect on the development and function of the HPA axis, which is a major part of the neuroendocrine system and thus provides humans with the ability to adapt to stress (Yılmaz & Gökmen, 2019).

Bacterial-regulation mechanisms play an important role in determining the local GI and circulating Trp availability for the host. Therefore, these factors determine serotonin synthesis, demonstrating the influence of gut microbiota on both the ENS and CNS neurotransmission, with implications for GI processes and neurological processes (Jenkins, Nguyen, Polglaze, & Bertrand, 2016). Gut bacteria can also synthesise serotonin (Agus, Planchais, & Sokol, 2018), which may affect either receptors within the gut or extra intestinal sites after luminal uptake into the portal circulation (Lyte, 2014). Likewise, microbiota can synthesise phenolic and indolic compounds, which can promote GI 5-HT synthesis from Trp (Gao et al., 2018).

Moreover, there is emerging preclinical evidence that many of the gut-brain communication pathways could be prone to dietary modulation, especially those related to the intestinal microbiota. This likely suggests the potential regulatory role of diet on the gut-brain axis and its associated disorders, including the chronic conditions that are so commonly comorbid with mental disorders (Bear et al., 2020; Berding et al., 2021).

4. The influence of milk bioactive components on 5-HT signalling

Milk proteins and their bioactive peptides can induce changes in certain neurological and intestinal processes regulated by the serotonergic system, either by alterations in 5-HT synthesis or through the interaction of dairy derivatives or milk-derived 5-HT with some serotonergic receptors. Furthermore, these bioactive compounds can also regulate the serotonergic system indirectly, through the modulation of intestinal microbiota. Therefore, a potential direct link is made between diet and brain/intestinal functions involving 5-HT signalling (Fig. 2).

4.1. Direct regulation of the serotonergic system

Bioactive milk proteins affect central and intestinal 5-HT synthesis due to their Trp content, which unlike 5-HT, is able to cross the BBB (Otsuka et al., 2015). The rate of cerebral 5-HT synthesis is regulated by the transport of free Trp across the BBB and depends strongly on diet composition. Large neutral amino acids (LNAAs) such as branched-chain amino acids (BCAA), compete with Trp in crossing the BBB through the L-system transporter. Therefore, a decreased plasma free Trp/BCAA ratio reduce the uptake of Trp, and subsequently, 5-HT synthesis in the brain (Cheng et al., 2016). Trp occurs in a wide range of dietary proteins, but some protein sources are richer in Trp than others are, so they may influence 5-HT signalling differently. Milk proteins are good sources of this amino acid; however, whey proteins possess more Trp than do caseins (Nongonierma & Fitzgerald, 2015). Consequently, some authors have found that α-lactalbumin (α-LA) has the highest ratio of Trp over its competitor amino acids (Orosco et al., 2004), so consuming a protein-based meal containing α-LA increases the Trp ratio by about 50%, thereby enhancing central and peripheral 5-HT synthesis and potentially enhancing 5-HT signalling (Fernstrom et al., 2013; Layman, Lönnerdal, & Fernstrom, 2018). This effect is especially interesting for CNS 5-HT synthesis, in which diet-induced changes in 5-HT levels occur mostly due to variations in this ratio. Centrally diminishing the 5-HT neurotransmitter induces a depressive state and an increased carbohydrate-rich or sweet food intake. These alterations are observed much more frequently in people with recurring mental disorders as anorexia or depression, which make them much more susceptible to alterations of the serotonergic system. However, serotonergic vulnerability might also occur in healthy individuals with chronic stress experiences because acute stress increases cortisol and serotonin in a compensatory manner. In the long term, high concentrations of cortisol decrease the sensitivity of 5-HT receptors, causing a decrease in 5-HT signalling, which may lead to greater susceptibility to changes in behaviour related to the serotonergic system (Verschoor, Finlayson, Blundell, Markus, & King, 2010). Therefore, the detrimental effects of decreased serotonin signalling on mood and eating behaviour might be moderated by an increase in CNS 5-HT, but earlier studies have shown that for mood and cognitive benefits, the ratio between Trp and other LNAAs must be increased by approximately 40% (Mitchell et al., 2011). Thus, considering that α-LA greatly increases the Trp ratio, it is suggested that the use of this protein in the diet could act as a precursor to the neurotransmitter serotonin, increasing brain serotonin activity. In this sense, some studies indicate a potential normalising effect of α-LA on the seasonal affective disorder (Feurté, Gerozissis, Regnault, & Paul, 2001), mood, depression, anxiety (Orosco et al., 2004), stress, cognitive function and sleep quality (Nongonierma & Fitzgerald, 2015).

Summarising these studies, it is clear that administration of large doses of tryptophan can alter brain serotonin levels and enhance mood. However, the influence of Trp-rich proteins on mood and other central serotonin related functions remains speculative, especially considering that 1%–2% of Trp in-take is converted into serotonin, with 95% of serotonin synthesised outside the CNS and degraded through the kynurenine pathway. Additionally, it is possible that the enhancing effect of α-LA observed in these studies is too small due to the short period studied. Consequently, further long-term studies with higher purified α-LA are needed to assess the specific described effects of this protein due to its Trp content (Layman et al., 2018).

Besides these effects, α-LA reduces prolactin and cortisol concentrations, so it improves mood and reduces the preference for sweet food individuals (Verschoor et al., 2010). Similarly, diverse studies of nematode *Caenorhabditis elegans* indicate that other whey proteins such as lactoferrin could promote serotonin synthesis by overexpression of the cat-4 gene, which participates in *C. elegans* neurotransmitter biosynthesis (serotonin and dopamine).

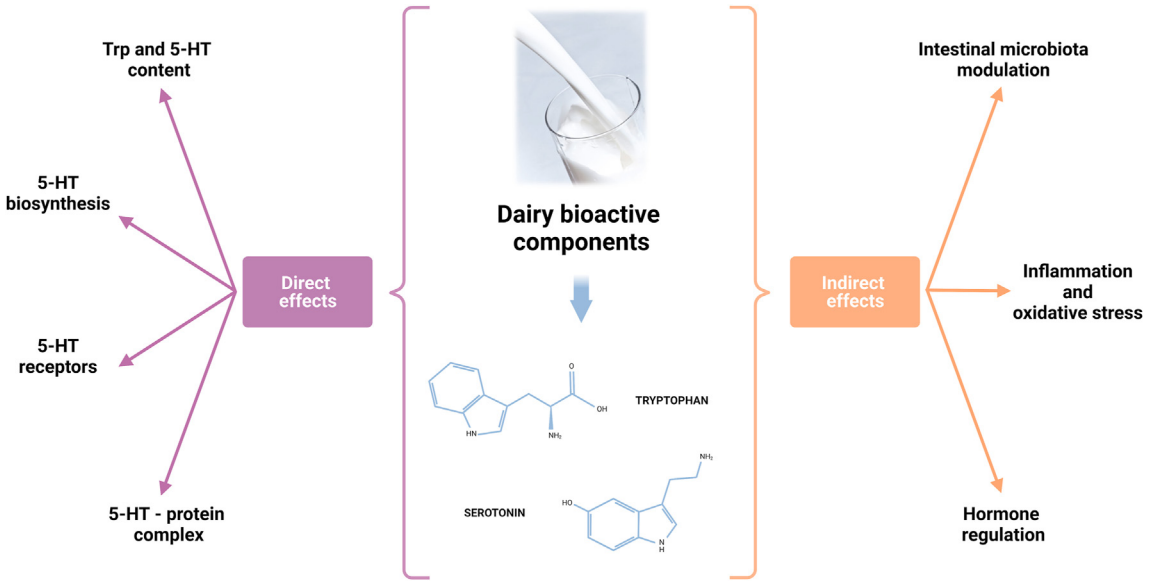

Fig. 2. Effects of dairy-derived bioactive components on the serotonergic system. Milk-derived bioactive proteins can regulate the 5-HT signalling directly via their tryptophan (Trp) and 5-HT content, which determine 5-HT biosynthesis and the activation of 5-HT receptors, respectively. In addition, some milk proteins can interact with several 5-HT receptors and endogenous 5-HT forming complexes to modify circulating 5-HT levels. These bioactive components regulate the serotonergic system indirectly in three ways: through the modulation of intestinal microbiota, because it is involved in 5-HT system along the gut–brain axis, and by modulating the activity and expression of PRRs, cellular oxidative status and inflammatory processes, where the 5-HT system is altered. Moreover, some dairy-derived bioactive components also alter the concentration of some hormones related with the 5-HT system such as cortisol, prolactin and GLP-1.

Interestingly, this gene is orthologous of the human GTP cyclohydrolase I, which participates in the synthesis of the tetrahydrobiopterin (BH4, THB), a cofactor of the Trp hydroxylase used in 5-HT biosynthesis (Martorell et al., 2017). However, further studies in *C. elegans* would be of interest to confirm the role of lactoferrin in 5-HT biosynthesis. Finally, clinical trials would also be necessary to confirm these results.

Other mechanisms by which dairy-derived dietary compounds influence the 5-HT system include their bioactivity on 5-HT receptors. Unlike α-LA, ingestion of a protein based meal consisting of casein decreases extracellular 5-HT in the brain because its Trp content is low compared with that of other LNAAs (Orosco et al., 2004). Nevertheless, some casein-derived bioactive peptides bind central 5-HT receptors, such as β-casomorphin-7, which binds and antagonises 5-HT2 receptors (Sokolov et al., 2005). The activation of the 5-HT2C receptor stimulates satiety. Therefore, its activation by some bioactive peptides could be very interesting as an antiobesity strategy (Schellekens et al., 2014).

Although it is still unknown how intestinal 5-HT is involved in food intake regulation, some studies hypothesised that casein could also regulate satiety through the stimulation of 5-HT release from EC cells, which would enhance the secretion of the satiety-inducing hormone GLP-1 via a serotonin receptor in the enteroendocrine cells. The 5-HT1B receptor might be a potential candidate, but further studies are needed to identify the specific serotonin receptors involved and the magnitude of serotonin's contribution to serotonin-stimulated GLP-1 release (Ripken et al., 2016).

Other studies have indicated that some milk proteins such as β-LG can interact directly with endogenous 5-HT and its derivatives, forming complexes. This could imply a peripheral control of 5-HT's quantity and action on the gut and other peripheral organs such as liver, where some metabolic functions depend on circulating 5-HT (Taheri-Kafrani et al., 2011).

Finally, several studies have shown that milk from various species, including bovine milk, contains such significant quantities of 5-HT that it might activate some peripheral 5-HT receptors, i.e., activating the 5-HT_3 receptor in the GI tract. Because this receptor is related to the control of vomiting, its activation at the GI level would exert an antiemetic effect without the need to activate this receptor centrally (Gallegos-Perez et al., 2014). In this sense, other fermented dairy products such as yoghurt and kefir would also contain significant amounts of serotonin and Trp (Yılmaz & Gökmen, 2019).

4.2. Indirect serotonergic system regulation through intestinal microbiota

Microbiota participate in serotonergic signalling through the gut–brain axis, and their modulation via diet could regulate 5-HT metabolism and improve 5-HT related gut–brain disorders. Therefore, we assess how some dairy-derived bioactive peptides affect human health and mood by inducing changes in gut microbiota (Yılmaz & Gökmen, 2019).

Many authors suggest that casein and whey milk exert a positive effect on gut health, providing a prebiotic effect. As mentioned previously, casein cannot induce changes in serotonergic signalling due to its low Trp content. However, it contains enough of this amino acid to induce changes in the microbiota, as happens with whey. This probably occurs because Trp is the main substrate for bacteria to produce phenolic and indolic compounds (Wang et al., 2019), which stimulate 5-HT synthesis via EC cells. Beside this, whey's main fermentable substrates are lactose, peptides and other amino acids, which give rise to other bacterial metabolites such as SCFAs, which are important mediators of the interaction between gut microbes and hosts (Sánchez-Moya et al., 2017), and are also connected to 5-HT synthesis via EC cells. Nonetheless, diverse reports have shown that some whey proteins such as lactoferrin can exert a bacteriostatic effect against some *Clostridium* species such as *Clostridium ramosum* (Teraguchi et al., 1995), a bacterium that stimulates 5-HT secretion from EC cells through some of their metabolites, especially deoxycholate and SCFAs (Mandić et al., 2019). In addition, lactoferrin seems to restore the normal

microbiota after antibiotic treatment (Bellés et al., 2022). Milk contains bioactive molecules with important functions such as defensive proteins. In this way, lactoferrin, whey, and buttermilk can reduce the oxidative stress induced by LPS, and alter expression of TLRs receptors (Buey et al., 2021), suggesting that milk-derived bioactive components can also modulate the serotonergic system indirectly through cellular oxidative status and the innate immune system (Latorre et al., 2014, 2016).

Continuing with whey protein, some experimental studies have shown that a diet containing prebiotics and bioactive milk proteins such as lactoferrin, fed in early life, may promote brain development, sleep and learning, and reduce stress-induced anxiety (Mika et al., 2018). This multi-ingredient diet can increase some probiotics such as *Lactobacillus* spp. and gene expression for markers of neuronal plasticity (serotonin receptors) and activity (the immediate early gene *cfos*) in the prefrontal cortex, and it can decrease activity-related gene expression in the amygdala. The changes in the genetic expression of *cfos* and some serotonergic receptors such as 5-HT1A and 5-HT2C, are positively correlated with diet-induced increases in *Lactobacillus* spp., suggesting again that the diet's impact on CNS is potentially related to its impact on the gut microbiota (Mika et al., 2018). Some reports support the idea that early development is characterised by swift neural maturation and thus nutrients ingested throughout such periods may have a greater influence over neuronal structure and function (Williams et al., 2016).

Currently, most species of bacteria that are capable of de novo 5-HT synthesis remain unknown. However, the fact that *Escherichia coli* can synthesise serotonin is widely known (Knecht et al., 2016), so controlling its growth through diet could be an interesting way to regulate plasma serotonin and central Trp levels. Some bioactive milk peptides derived from casein such as casocidin-I and caseinomacropeptides, as well as other lactoferrin peptides such as lactoferrampin, display inhibitory activity against *E. coli* (Mohanty et al., 2016). Therefore, these peptides could reduce plasma serotonin levels and increase central Trp and 5-HT synthesis, thus improving some processes regulated by the central serotonergic system such as mood, cognitive function (Jenkins et al., 2016), and sleep efficiency and reducing sleep latency (Layman et al., 2018). Further exploration of possible gut microbiota manipulations through dairy proteins and peptides by modulating Trp metabolism pathways or reshaping the microbiota by targeting specific bacteria populations, will provide novel insights into the development of individual targeted approaches that can be a promising approach to preventing and treating gut–brain axis disorders such as GI functional disorders (e.g., IBS or IBD) and neuropsychiatric diseases.

5. Discussion

The production of functional foods containing bioactive peptides is aimed at either enhancing their bioavailability from natural sources or creating novel foods via the addition or fortification of isolated or enriched fractions of bioactive peptides (Korhonen, 2011). The digestion of milk proteins by enzymatic hydrolysis is one of the most frequent industrial processing techniques used for the generation of protein hydrolysates with physiological functions, while improving their absorption and reducing antigenicity and allergenicity. Hence, recent findings indicate that these milk protein hydrolysates constitute a potential source of highly functional ingredients (Mudgil & Barak, 2019). However, some authors assert that milk protein hydrolysis does not always allow the achievement of superior bioavailability compared with unhydrolysed proteins. This could be linked to the fact that the bioactive peptides are not optimally released during hydrolysis due to food matrix effects, may be degraded during GI digestion, or have low intestinal permeability. In this context, the development of milk protein hydrolysates capable of improving human health may require a targeted release of particularly potent specific bioactive peptides that are more likely to be bioactive in vivo (Nongonierma & FitzGerald, 2018).

Besides hydrolysis, other industrial treatments such as homogenisation, high temperature or ultra-high-temperature sterilisation have certain negative effects on milk components such as proteins and other bioactive substances (Tunick et al., 2016). Therefore, the effects of food processing must be taken into account because thermal processes may affect the concentrations of the neuroactive compounds (Yılmaz & Gökmen, 2019), and induce important structural and chemical changes in milk proteins. This is a challenge for the dairy industry, which has already carried out research on the application of nonthermal processing technologies such as membrane filtration (Haug et al., 2007).

Compared with milk proteins, carbohydrates and fats also modulate 5-HT signalling, but they have different effects, especially on mood and simple cognitive functions. These effects are related to this nutrient's influence on the synthesis of brain serotonin and other neurotransmitters such as catecholamines, which are mediated by the ratios of plasma Trp and tyrosine (Tyr) to the other LNAAs (Fischer, Colombani, Langhans, & Wenk, 2002). Carbohydrate ingestion does not change the levels of circulating Trp, but it further increases the Trp/LNAA ratio in the plasma (Richard et al., 2009; Wurtman et al., 2003) because insulin secretion facilitates the transportation of branched-chain amino acids from the bloodstream to peripheral tissues, thus increasing the relative availability of Trp for transport into the brain (Yılmaz & Gökmen, 2019). Based on these findings, a diet rich in carbohydrates can relieve depression and elevate mood via increased central serotonergic activity. Consequently, dieters tend to become depressed because decreased carbohydrate intake lowers their serotonin levels. However, carbohydrate consumption often leads to obesity, especially among people who are stressed, depressed, or trying to give up smoking. Nicotine increases brain serotonin secretion, and withdrawal from it leads to depression (Singh, 2014). This can be considered a disadvantage with respect to protein consumption as a stimulant of the brain serotonergic system.

Dietary fatty acids might also affect the metabolic fate of Trp and its availability for uptake into the brain. A diet high in polyunsaturated fatty acids such as ω3 fatty acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) affects serotonergic transmission and has been associated with the prevention of some psychiatric ailments and with a high capacity for coping with stress (Höglund, Øverli, & Winberg, 2019). The mechanisms for this antidepressive action of ω3 fatty acids are related to their capacity to suppress the production of some inflammatory cytokines. EPA has a cytokine-suppressing effect, and DHA reduces the expression of TNF-α and interleukin-6. This, in turn, increases Trp influx to the brain and, subsequently, brain 5-HT synthesis (Schmidt, Kirkby, & Lichtblau, 2016).

Similarly, an increase in plasma nonesterified fatty acids (NEFAs), also increases the free circulating Trp available for uptake into the brain, as these displace Trp from its binding to albumin. Increased circulating NEFA levels come from lipolysis, which can be induced by the release of catecholamines in cases of acute stress (Chaouloff, 1993). Since the literature focuses on how these dietary components affect central 5-HT signalling, further studies investigating carbohydrates and fats that regulate the peripheral 5-HT system are warranted to learn its possible involvement in GI processes mediated by peripheral 5-HT.

6. Conclusions

Multiple mechanisms of the microbiota–gut–brain axis are prone to dietary modulation and have been suggested to underlie the effect of diet on gut and brain in some investigations. While the effects of probiotics, prebiotics and general dietary patterns have been more widely studied, investigations into certain isolated foods are scarce. In this sense, bovine milk proteins and bioactive peptides have attracted the interest of researchers as health promoting functional foods because of their numerous biological properties.

Although the physiological significance of several of these substances is not yet fully understood, proteins and bioactive peptides derived from bovine milk are believed to be health enhancing components that could be used to regulate brain/intestinal physiology through its impact on 5-HT system, since 5-HT is involved at every level of the gut–brain axis, with a wide range of roles that are essential to life. Milk peptides have the dual benefit of a direct impact on the 5-HT system and an indirect effect via the gut microbiota. These bioactive compounds can directly influence the serotonergic system either by alterations in 5-HT synthesis or through their interaction with serotonergic receptors. Furthermore, some peptides might influence the 5-HT synthesis by gut bacteria. Thus, dietary interventions targeting neurobiological and GI processes regulated by the 5-HT system might be a viable and an economically cheaper treatment or co-adjuvant strategy for serotonin-related gut-brain axis disorders such as depression and IBD.

To drive the development of microbiota–gut–brain axis targeted human interventions, detailed information about which microorganisms are involved in this axis would facilitate a more precise study of milk proteins and peptides that could be used to selectively modify the activity and growth of the intestinal microbiota. Likewise, the impact of diet on microbiota is highly personalised and dependent on the underlying microbiota. Consequently, in some cases, personalised nutrition could be implemented to improve and treat serotonin-related gut–brain axis disorders.

One of the major future challenges is determining how to best formulate milk-derived bioactive compounds as food supplements so that they have high intestinal permeability and can withstand the harsh environment of the GI tract. In addition, it must be ensured that their effects are not neutralised by antagonistic bioactive compounds simultaneously ingested in foods. These challenges and the limited evidence from intervention studies in humans makes it difficult to provide evidence-based recommendations for the use of milk bioactive peptides in improving gut-brain axis disorders. This highlights the imperative need for additional research to further our understanding of unravel complex interactions and causal relationships among milk peptides and the microbiota–gut–brain axis.

Declaration of competing interest

None.

Data availability

No data was used for the research described in the article.

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