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UPDATE ARTICLE

Opioid peptides and gastrointestinal symptoms in autism spectrum disorders

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Autism spectrum disorders (ASDs) are characterized by deficits in the individual's ability to socialize, communicate, and use the imagination, in addition to stereotyped behaviors. These disorders have a heterogenous phenotype, both in relation to symptoms and regarding severity. Organic problems related to the gastrointestinal tract are often associated with ASD, including dysbiosis, inflammatory bowel disease, exocrine pancreatic insufficiency, celiac disease, indigestion, malabsorption, food intolerance, and food allergies, leading to vitamin deficiencies and malnutrition. In an attempt to explain the pathophysiology involved in autism, a theory founded on opioid excess has been the focus of various investigations, since it partially explains the symptomatology of the disorder. Another hypothesis has been put forward whereby the probable triggers of ASDs would be related to the presence of bacteria in the bowel, oxidative stress, and intestinal permeability. The present update reviews these hypotheses.

Keywords: Autistic disorder; probability theory; opioids; intestinal bacteria; oxidative stress; bowel permeability

Introduction

Autism spectrum disorder (ASD) is characterized by stereotyped behavior and a deficiency in the individual's ability to socialize, communicate, and use imagination.[1] Although the disorder is associated with neural development, its true causes remain to be clarified. Recent evidence supports the hypothesis of a complex and highly heterogenous genetic etiology, and points to a combined effect between the environment and various different genes.[2] As a consequence, phenotypes in ASD appear to be manifold.

Many autistic individuals suffer from gastrointestinal problems[3] such as abnormalities of the bowel mucosa, dysfunctions associated with selective permeability, and significant differences in composition of the gut microbiota.[4] Individuals with ASD also exhibit an imbalance in immune response.[3] Since the gastrointestinal tract has direct connections with the immune system, alterations in the gastrointestinal tract may generate alterations in the immune system.

In an attempt to explain the pathophysiology involved in autism, Panksepp et al.[5] conducted a pioneering experimental study with rats and reported a decrease in social cohesion with an increase in opioid peptides in the organism. Recent studies investigated the role of opioid peptides and intestinal permeability,[6] as well as alterations in the expression of certain genes.[7] Heberling et al.[2] suggested that certain gut bacteria, oxidative stress and changes in intestinal permeability could be involved in the etiology of ASD.

Factors related to nutrition, such as gluten and casein intolerance, are under investigation, as they are associated with various gastrointestinal symptoms. Thus, interventions have been implemented in which gluten and casein are excluded from the diet and probiotics and multivitamins are included.[4] This update article discusses current hypotheses that associate the etiology of autism with alterations in opioid metabolism and with gastrointestinal tract symptoms.

The opioid excess theory

Panksepp et al.[5] found that an excess of casomorphin, an opioid-like peptide fragment of milk casein, induced social isolation and apathy in animals. Panksepp[8] then proposed the opioid excess theory to explain the pathogenesis of ASD and advocated that opioid peptides released from gluten and casein could pass through the mucosa and cross the blood-brain barrier, reaching the central nervous system (CNS) and affecting brain function.

Food-derived bioactive peptides are protein fragments originating from enzymatic hydrolysis and proteolysis induced by microorganisms.[9] They consist of oligopeptides (three to 20 amino acids) with distinct functions and conformations.[10] Some bioactive peptides act directly on the gastrointestinal lumen, while others act on peripheral organs following absorption in the bowel, playing a role in reducing arterial blood pressure, modulating immune cells, and regulating nervous functions.[11] Factors involved

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in the absorption of these peptides include the inhibition of peptidases[12] and the permeability of the intestinal barrier.[13] Nevertheless, the opioid excess theory has been criticized due to the fact that no abnormal concentrations of opioid peptides had ever been detected in the plasma or in the CNS of individuals with ASD.[14]

Randomized controlled studies have been conducted to evaluate the efficacy of excluding gluten and casein from the diet of individuals with ASD.[15,16] Some results suggested positive effects on behavior and development; however, other studies failed to find any improvement in the intervention group.[17,18] Meta-analyses[5,19] recommended caution with respect to adopting this diet because of limited scientific evidence on its use. Furthermore, the dietary restrictions imposed may lead to more social rejection and stigmatization, as well as to deficits in these individuals' socialization and integration.

Hypothesis on the association among gut bacteria, oxidative stress, and intestinal permeability

The microbiota of the human gastrointestinal tract plays a central role both in health and in the generation of disease.[3] Anaerobic bacteria account for the production of acetic and propionic acid, in addition to short-chain fatty acids, particularly butyric acid, which supplies energy to the intestinal epithelial cells and consequently strengthens the immune system. The principal bacteria producing short-chain fatty acids are *Bacteroides*, *Lactobacillus*, and *Bifidobacterium*, which induce the production of anti-inflammatory cytokines, unlike *Clostridium*, which stimulates the proinflammatory cytokines. Bacteria of the *Clostridium* genus, such as *C. difficile* and *C. perfringens*, are major producers of toxins.[20]

In the intestinal microbiota of individuals with ASD, the amount of bifidobacteria is reduced and there is abnormal growth of *Clostridium* species.[3] Finegold et al.[20] identified *Desulfovibrio* as the prevalent organism in the intestinal flora of these individuals and found that it modifies the bacterial ecosystem, reducing the presence of important species such as *Bifidobacterium longum* and *B. pseudolongum*, which produce hydrogen sulfate, a gas that is toxic to humans.[3]

Sulfur metabolism

The oxidation of thiol (sulfhydryl) groups may play a central role in the development of ASD.[3] Meta-analyses suggest that there are deficiencies in the transmethylation/transsulfuration metabolism[21] associated with the sulfur amino acids methionine and cysteine.

The essential amino acid methionine must be available in the diet to enable cysteine synthesis to occur. Deficiencies in metabolic pathways will compromise the excretion of heavy metals, because cysteine is a limiting factor in the production of reduced glutathione, a natural antioxidant in the body.[22] In fact, various metabolic precursors involved in the synthesis of reduced glutathione are decreased in the plasma of individuals with ASD, suggesting that synthesis of this antioxidant may be compromised.[3,22] As a consequence of this metabolic inhibition, autistic individuals may be more sensitive to the toxic effects of heavy metals. Evidence of this was found in urine and blood samples from people with ASD. Compared to those of controls, blood samples from the individuals with ASD showed greater amounts of lead in the red blood cells, while their urine samples showed higher tin, tungsten, and lead levels.[23]

Transmethylation is also involved in the regeneration and conversion of methionine into *S*-adenosylmethionine, which is the most important enzymatic co-factor in the transfer of methyl groups for the biosynthesis of DNA, RNA, proteins, phospholipids, creatinine, and neurotransmitters.[22]

Intestinal permeability

The proper function of the intestinal mucosal barrier guarantees that the bowel counteracts the entry of microorganisms and molecules, thus preserving its ability to absorb nutrients.[12] The secretion of mucins, immunoglobulin A, and antimicrobial peptides strengthens the mucosal barrier in the outer mucus layer, while internally, the immune cells provide this protection.[12] The mucosal barrier has physical, biochemical, and immune characteristics that allow it to secure the flow of substances through the paracellular pathway, which is associated with transportation through the intercellular space.[24]

Intestinal permeability to small water-soluble molecules is determined by the tight junctions, which open and close constantly in response to stimuli such as diet, humoral or neuronal signaling, and through inflammatory mediators.[25] Although the tight junctions are represented by a complex of more than 50 proteins, there is evidence that the family of claudins (transmembrane proteins) and zonulin[26] are involved in the regulation of selective permeability, including size, electric resistance, and preference for ionic charge.[27] The delicate nature of this balance is interrupted in pathologic states. It has been reported that zonulin expression increases in autoimmune conditions and is associated with dysfunction of tight junctions. Physiologically, however, exposure of the bowel to bacteria and to gluten triggers zonulin release.[26] Some studies suggest that the function of the epithelial barrier is damaged in ASD,[13] while others have reported that individuals with ASD are prone to gluten allergy[17]; therefore, it would be reasonable to presume that zonulin could be involved in this process.[26]

Heberling et al.[3] suggested that the pathogenesis of ASD was associated with the relationship between three factors: oxidative stress with a subsequent metabolic sulfur deficiency, an abnormal growth of gut bacteria, and an increase in intestinal permeability. The metabolic sulfur deficiencies associated with an imbalance in the composition of gut bacteria, principally with a proliferation of *Clostridium* and/or *Desulfovibrio*, together with a reduction in bifidobacteria,[3] would explain the association between the first two factors. In addition, the metabolic sulfur deficiency would be self-perpetuating, with decreased total and reduced glutathione levels resulting from the decline in bifidobacteria.[3] A dysfunctional intestinal mucosa would, in turn, facilitate the absorption of toxins, bacterial products,

lymphocytes, proinflammatory cytokines, and neurotransmitters, which would reach the bloodstream, cross the blood-brain barrier, and cause neuroinflammation.[3]

Although the interactions of the brain, bowel, and microbiome are multifactorial and not yet completely clarified, the vagal system functions as a communication channel between the microbiota and the brain.[27] In view of the aforementioned scientific evidence, it is suggested that the microbiome may play an important role in the CNS, both in promoting health and in the genesis and maintenance of pathologic states. There are direct and indirect interactions between the gut-brain axis and microbiome, which may be associated with a new concept of integrative physiology in which associations occur between the neural, immune, and endocrine systems, as well as with nutrients and immune markers of the CNS and gastrointestinal tract.[28]

The mechanisms used by pathogens to influence host behavior have been known for decades; however, evidence is growing for direct, noninvasive interactions with the neurophysiological system. The ability of microorganisms to produce and recognize neurochemical compounds structurally similar to those produced by the host's nervous system explains how they are able to affect behavior through a non-infectious and, possibly, non-immune-mediated pathway.[29] Looking at the microbiome from the standpoint of microbial endocrinology, specific pathways are involved in which microorganisms affect behavior, permitting a new approach for the treatment of mental illnesses by modulating the microbiome-gut-brain axis.[30] Studies conducted to investigate these effects have shown that cytokines and inflammatory mediators have known neuronal targets, both in the CNS and in the enteric nervous system (Meissner's plexus and Auerbach's plexus).[30] The fact that bacteria produce neuroendocrine hormones also suggests that the interaction of the microbiome may go well beyond the bacterial neuroendocrine interactions that occur in infectious diseases.

Conclusion

Current literature does not support the opioid excess theory, since abnormal opioid peptide levels have never been found in the CNS of individuals with ASD. Nevertheless, oxidative stress in individuals with ASD may be a consequence of metabolic sulfur deficiency, abnormal gut bacteria growth, and increased intestinal permeability, thus suggesting a possible correlation between gastrointestinal abnormalities and symptoms of ASD. These data suggest that it may be possible to improve autism-related symptoms by modulating the microbiome-gut-brain axis in individuals with a specific phenotype. Although the evidence to support opioid-free diets (gluten-free, casein-free) is limited and weak, dietary restrictions should only be introduced after gastrointestinal symptoms have appeared or intolerance or allergy to these foods has been diagnosed.

Disclosure

The authors report no conflicts of interest.

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