ISSN: 3065-4874
Review Article
Journal of Psychiatry and Neurochemistry Research

The Resemblance between the Propionic Acid Rodent Model of Autism and Autism Spectrum Disorder

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Submitted: 30 September 2023 **Accepted:** 07 October 2023 **Published:** 13 October 2023

https://doi.org/10.63620/MKJPNR.2023.1014

Citation: *Joon, P., Kumar, A., & Parle, M. (2023). The Resemblance between the Propionic Acid Rodent Model of Autism and Autism Spectrum Disorder. J of Psych and Neuroche Res 1(3), 01-08.*

Abstract

Autism spectrum disorder is a complex, lifelong, heterogeneous, multi-factorial developmental disability with a dynamic set of metabolic, mitochondrial, immune, neuroinflammatory, and behavioral abnormalities affecting several body parts. Autism Spectrum Disorder (ASD) is manifested by cognitive, behavioral, and social deficits with early childhood onset. Appropriate animal models that can mimic core symptoms of Autism are difficult to develop, although essential to explore the pathogenesis of ASD and discover potential medicines useful in this disorder. Propionic acid (PPA) is an intermediary product of cellular fatty acid metabolism found in the gut. Furthermore, PPA is an end product produced by the intestinal tract, skin, and oral mucosa bacteria. In addition to its endogenous synthesis, PPA is naturally present in various foodstuffs as a preferred preservative. Although PPA may be beneficial at appropriate levels, excessive PPA appears to have some adverse effects on health and behavior. PPA can readily cross the gut-blood, blood-brain, and placental barriers. PPA particularly gets concentrated intracellularly, producing deleterious effects on the developing brain. This becomes noteworthy in the context of ASD since PPA affects cell signaling, neurotransmitter synthesis, mitochondrial function, lipid metabolism, immune function, neuroinflammation, and gene expression. After administration of PPA by different routes in rodents, the observations suggest that PPA may be linked to the behavioral, neuropathological, and biochemical abnormalities observed in ASD. There is a possibility that Propionic acid could be the intrauterine candidate responsible for causing harm to the unborn child, resulting in the development of autism-like disorder. This review article elucidates a complete picture of Propionic acid's effects and mechanism of action when administered to rodents. The authors have examined the validity and utility of the PPA model of Autism in this review article. A thorough analysis of the data available in the literature from preclinical studies as well as the clinical reports justifies the utility of the PPA rodent model of Autism as a tool in neurochemistry and experimental pharmacology.

Keywords: Autism, Propionic Acid, Rats, Intracerebroventricular, Social Deficits

Introduction

Autism spectrum disorders (ASD) are a family of disorders characterized by stereotypic and restrictive patterns of behavior, deficits in social interactions, and impairments in language development and communication skills [1]. Several recent studies have indicated that interactions between genetic, metabolic, immunological, gastrointestinal, environmental, and behavioral factors may be associated with the pathogenesis of ASD [2, 3]. ASD occurs concurrently with other neurological conditions, including epilepsy and attention deficit hyperactivity disorder [4-6]. There are presently no reliable biomarkers for the diagnosis of ASD, and the etiology of ASD continues to be unclear. While the causal factors of ASD remain unknown, there is strong evidence for a multi-genetic role in ASD [3, 5]. However, research also indicates that environmental and gut-related factors may be important and that Autism may be a multi-system disorder affecting metabolic, immune, environmental, genetic, and gastrointestinal systems, given the diverse symptoms [7-11]. Well-defined

animal models that can exhibit core symptoms of Autism thus become essential to identify potential medicines useful in this disorder. It is difficult to address questions regarding causative factors in ASD-suffering patients, so animal models may greatly help [10]. The authors propose to examine possible environmental triggers of ASD, such as bacterial or gut metabolites. There is a possibility that Propionic acid (PPA) may be the intrauterine candidate involved in the development of some types of childhood disorders [2]. Thus, it appears that PPA may be playing a crucial role in the clinical pathogenesis of ASD. Therefore, administering PPA to rodents might represent a useful model to investigate the potential of new medicines effective in managing ASD and throw more light on the pathogenesis of the condition.

Why Propionic Acid?

Propionate and other short-chain fatty acids (for example, butyrate and acetate) are produced in the body during normal cellular metabolism [2]. Propionic acid (PPA) is an intermediary product in cellular fatty acid metabolism found in the gut, along with other short-chain fatty acids such as acetate and butyrate. PPA is an end product produced by bacteria of the intestinal tract skin and oral mucosa [12-14]. In addition to its endogenous synthesis, PPA is naturally present in various foodstuffs and is commonly used as a preservative in refined wheat and dairy products [15, 16]. PPA-producing enteric bacteria include unique Clostridial, Desulfovibrio, and Bacteriodetes species isolated from patients with regressive ASD [2]. Under normal circumstances, these short-chain fatty acids are primarily metabolized in the liver. Under pathological circumstances, if there are genetic and/or acquired aberrations in metabolism, higher than normal levels of short-chain fatty acids can be present in the circulating blood and can cross the gut-blood and blood-brain barriers passively and/or actively via high-affinity transporters [2, 15]. PPA can readily cross the gut-blood, blood-brain, and placental barriers. PPA particularly gets concentrated intracellularly, producing deleterious effects on brain development and function [15]. This could be important in the context of ASD since PPA is known to affect cell signaling, neurotransmitter synthesis and release, mitochondrial function/CoA sequestration, lipid metabolism, immune function, gap junction modulation, neuroinflammation, free radical formation, and gene expression, all of which have been implicated in ASD [2]. Propionic acid (PPA) is a short-chain fatty acid (SCFA) that has been implicated as a possible gut-derived environmental factor in ASD reaching womb of the pregnant ladies [10, 17, 18]. During the early stages of pregnancy, increased consumption of PPA-rich processed foods combined with pre-existent dysbiosis (loss of beneficial bacteria) may lead to the accumulation of PPA in the maternal GI, travel through general circulation, cross the placental barrier, and interfere with neural differentiation [18]. Although PPA may be beneficial at appropriate levels excessive PPA appears to negatively affect health and behavior [19]. Several inherited and acquired conditions, such as propionic/ methylmalonic acidemia, biotinidase/holocarboxylase deficiency, ethanol/valproate exposure, and mitochondrial disorders, are known to result in elevations of PPA [16, 20]. PPA elevation at the intracellular level appears to be a key factor responsible for developmental delay, regression, seizure, movement disorder, gastrointestinal symptoms, and cognitive decline, all of which bear similarities to the symptoms observed in autism spectrum disorders. The research findings and/or clinical reports suggest that PPA may be linked to the behavioral, neuropathological, and biochemical abnormalities observed in ASD [16].

Toxicity with Propionic Acid

Propionic acid is commonly used as a preservative in several food items because of its antibacterial properties. The Food and Drug Administration (FDA) has certified Propionic acid as a "Safe" preservative in human beings. When ingested orally, propionic acid causes gingival inflammation, sore throat, and systemic acidemia. However, direct contact of PPA with the skin leads to local redness, pain, and burns due to tissue damage. Inhalation of PPA leads to inflammation of the nasal tract. Furthermore, undiluted PPA (greater than 0.26M), in direct contact with human eyes, produces serious adverse effects such as corneal damage leading to total blindness. In clinical settings, it has been observed that Propionic acid levels are elevated in blood, urine, and feces collected from autistic children [21].

Relevance of PPA in Autism

Autism is a neurodevelopmental, heterogeneous, complex disorder that cannot be simply attributed to genetics or environmental adversities [1, 22]. Rather, recent research examines ASD as a dynamic encephalopathic condition involving immune, digestive, and metabolic dysfunction that may be exacerbated by environmental triggers in genetically sensitive subpopulations [3, 20]. Recently, a huge body of evidence has proposed a connection between dietary factors and Autism. The human digestive tract is a host to a complex array of intestinal bacterial florae, both harmful and protective, that produce an astounding number of bioactive metabolic products capable of entering systemic circulation and central nervous system while having profound effects upon host metabolism, immune function, and gene expression [23]. Notably, PPA is produced by many of these gut bacteria via the breakdown of dietary carbohydrates and amino acids. The Clostridial and Desulfovibrio bacteria species are particularly relevant and have been proposed as an infectious cause of ASD [24, 25]. Clostridial species are a family of heterogeneous anaerobic, spore-forming gram-positive rods that are major gut colonizers in early life and are producers of PPA.

Interestingly, spore-forming anaerobes and microaerophilic bacteria, particularly from Clostridial species, have been shown to be elevated in ASD patients [16]. Desulfovibrio, a gram-negative, aero-tolerant, non-spore former resistant to most common antibiotics and producing PPA, has been isolated from ASD patients [25]. An association between long-term antibiotic use, abdominal discomfort, and the onset of ASD symptoms even after normal development has been observed [25]. These findings raise the possibility that gut-born factors may influence brain function and symptomology in ASD patients. PPA is known to have some direct effects on gastrointestinal physiology. PPA increases colonic smooth muscle contraction, dilates colonic arteries, activates mast cells, increases the release of serotonin from gut enterochromaffin cells, and reduces gastric motility [15]. Thus, PPA appears to possess the necessary properties to interfere with gastrointestinal activity, like the abnormalities observed in ASD. To substantiate this hypothesis, feedback from parents of ASD children suggests that symptoms increase when their children ingest refined food products that either contain PPA as a preservative or provide high carbohydrates for bacterial fermentation to produce PPA.

Furthermore, there are reports indicating improvements in the behaviors of ASD children following the elimination of these PPA-elevating products from the diet [16, 26]. Moreover, symptoms associated with propionic acidemia are similar to the clinical signs observed in ASD children [27]. Thus, elevated levels of PPA could serve as a gut-derived factor reaching the uterine environment during prenatal development of the child, evoking adverse effects in the womb.

Can PPA Serve as an Animal Model of Autism?

Autism is a developmental disorder recently defined across three domains, viz., deficits in social interaction, communication difficulties, stereotypic behavior, and restricted interests. Albeit autism spectrum disorder is uniquely human, animal models can provide insight into the underlying pathogenesis of this disorder and facilitate the development of potentially beneficial therapeutic agents. With the help of a thorough understanding/observation of behavioral patterns seen in ASD children, most of the core characteristics of Autism, such as stereotypic behavior, impaired social interaction, and cognitive decline, can be modeled in rodents. Therefore, developing an animal model that allows the experimental examination of the influence of suspected endogenous and environmental agents evoking ASD-like symptoms becomes essential. PPA elevation at the intracellular level appears to be a key factor responsible for developmental delay, regression, seizure, movement disorder, gastrointestinal symptoms, and cognitive decline, all of which bear similarities to the symptoms observed in autism spectrum disorders. The research findings and/or clinical reports suggest that PPA may be linked to the behavioral, neuropathological, and biochemical abnormalities observed in ASD [16]. In light of the above, the diverse effects of PPA on rodents' behavior and cognitive functions, outlined in the preceding pages, can serve as a useful animal model of Autism.

Diverse Effects of PPA Related to Autistic Symptoms

PPA is a common preservative in food products that exacerbates ASD symptoms [17]. PPA evokes social deficits, abnormal behavioral patterns, and cognitive impairments in rodents of both sexes and different age groups when administered by multiple routes of administration, such as intracerebroventricular (ICV), intraperitoneal (i.p.), oral, and subcutaneous (s.c.), following different doses and frequency schedules. Studies administering intracerebroventricular PPA to rodents reported social abnormalities, cognitive impairments, and sensorimotor dysfunction [10]. Examination of brain tissue from PPA-treated rats has revealed reactive astrogliosis, activated microglia, oxidative stress, glutathione depletion, mitochondrial dysfunction, and alteration of phospholipid/acylcarnitine profiles, all of which are consistent with the findings in ASD patients [2, 10, 15, 28-31]. Administration of PPA, but not control compounds, produced short bouts of behavioral (hyperactivity, perseveration, object fixation, social impairments) and electrophysiological (seizure, caudate spiking) effects, coupled with biochemical (increased oxidative stress, reduced glutathione) and neuropathological (innate neuroinflammation) alterations in adult rats, consistent with those seen in ASD [7, 10, 15-17]. Recent findings with this model indicate infusions with PPA altered the brain acylcarnitine and phospholipid profiles [28]. Interestingly, the alterations in rat brain lipids showed similarities to those documented in blood samples of ASD patients [2].

• PPA Evokes Deficits in Social Skills

Impairments in social behavior and reciprocal interaction, including abnormal play behaviors and different forms of social contact, are among the hallmark symptoms of ASD [28]. To date, several studies have examined the effects of PPA on social behavior in adolescent and adult rats [28, 30]. In the initial studies, ICV injections of PPA (4 µl of 0.26 M solution or 1M solution, pH 7.5) exhibited deficits in the social behavior of rats as reflected by the greater mean distance apart, reduced time spent in close proximity to another rat, reduced playful interactions, and altered responses to playful initiations in an open field test [28, 32]. On the other hand, treatment with propanol, the alcohol analog of PPA, did not produce these social deficits. Furthermore, it was observed that adolescent rats treated with ICV injections of PPA preferred spending more time with a novel object than a novel rat compared to saline-treated animals in a novel rat versus novel object choice test [16]. Several studies have reported social abnormalities in Seizure-prone (FAST) rats [33, 34]. In particular, the FAST rat strain initiated more playful attacks and was more likely to defend against an attack than seizure-resistant (SLOW) rats [33]. FAST rats also displayed more evasive defense tactics that reduced the likelihood of prolonged interactions [33]. A single ICV injection of PPA was found to impair social behavior and induce astrogliosis in both Seizure-prone (FAST) and seizure-resistant (SLOW) rat strains [10]. These findings suggest that PPA can cause social abnormalities in rats consistent with the social deficits observed in ASD children. PPA administered intraperitoneally (500mg/kg) for 5 days to male rats exhibited a reduction in exploratory activity compared to control and a decline in time taken to approach another rat in an open field, indicating impairment of social interaction [2]. Whereas PPA administered at 500mg/kg for 5 days to female rats did not significantly affect social interaction parameters [21, 35]. PPA administered intraperitoneally at 250mg/kg for 17 consecutive days to 21-day-old rat pups evoked social deficits in the box chambers model [36]. A single intraperitoneal injection of PPA (250mg/kg and 500mg/kg) evoked hyposensitivity and produced significant dose-dependent reductions in startle response magnitude relative to control adult male rats [37]. Intraperitoneal injection of PPA (500mg/kg) for 7 days to 35 days in juvenile male rats evoked impaired social interaction and hypoactivity when tested using circular open field test [21, 38]. These findings involving PPA administration in different rat strains produce social deficits similar to ASD patients, providing further evidence for the robustness of the PPA model of ASD.

• PPA-Induced Cognitive Decline

The core symptoms of ASD include limited interests, repetitive patterns, ritualistic, stereotypic behavior, cognitive impairments, and deficits in reciprocal social interaction [1, 22]. In order to validate the PPA model of ASD, researchers have examined the effects of PPA on the cognitive ability of rodents using various behavioral models [29-30]. In the water maze test, the trained control animals could trace the hidden platform swiftly compared to trained PPA-treated animals. Furthermore, normal animals spent more time in the target quadrant where the remote platform was placed as compared to ICV-PPA-treated animals, indicating cognitive decline due to PPA administration [16]. Propionic acid (PPA) at 500 mg/kg/day administered

subcutaneously for 5 days produced significant impairment of learning and memory in the Morris water maze task and oxidative damage indicated by increased serum and brain levels of MDA [39]. The T-maze test, consisting of three arms inclusive of one closed arm and two open arms, has been employed to measure inhibitory avoidance representing learned fear. During inhibitory avoidance acquisition, the rat is placed at the distal end of the enclosed arm. The latency to leave this enclosed arm is measured immediately after the animal is released from the experimenter's hand. Therefore, the motivation for the animal to walk along the enclosed arm could not only be to explore the new environment but also to escape from the experimenter's hand. ICV-PPA-treated adolescent male rats exhibited restrictive interests and showed no motivation to reach the open arm, possibly out of unknown fear [16]. When the animals were exposed to the beam task, PPA-treated animals exhibited more slips and falls than the control group. These results indicate impairment in the learned beam walking skill, and enhancement of unusual behavior. Studies also examined restrictive interests and repetitive behaviors in PPA-treated adolescent rats in response to a group of novel stimuli objects [16]. PPA-treated adolescent rats approached and preferred to remain close to one particular object, ignoring two other objects placed in the same arena, in addition to showing more sniffing behavior in the novel object recognition test. These observations revealed the limited interest of the animals in exploring the entire environment [30]. In rats, ICV infusions of PPA (4 µl/infusion of 0.26 M, pH 7.4) twice a day for 7 consecutive days showed deficits in spatial memory in the water maze test. Following a recovery period of 1 week of no treatment, these animals restored normal spatial memory, indicating that the behavioral cognitive deficits caused by PPA were reversible [40]. Intraperitoneal administration of PPA at the dose of 250mg/kg for 3 consecutive days to 21 days old Wistar rat pups interfered with spatial memory when tested on the Y maze and produced fear as indicated by less time spent and reduced number of entries in open arms when tested using elevated plus maze [41]. The findings obtained from the water maze test, T-maze test, novel object recognition test, and sniffing behavior test revealed that PPA can increase stereotypic behavior, enhance restricted interests, exhibit learning deficits and memory impairments consistent with cognitive decline observed in children suffering from ASD [16, 29, 30].

• Complex Influence of PPA on Motor Function

Children suffering from ASD often exhibit motor-related abnormal behavioral patterns such as stereotyped movements, hyperactivity, gait disturbances, posture imbalance, frequent slips and falls, paddling motions of the limbs, circular movements of body limbs, impairments in fine motor ability, and atypical behavior [15-16, 34]. PPA administered at a dose of 500 mg/kg/day subcutaneously to pregnant female rats on gestational days 12–16 for a total of 5 injections gave birth to offspring of both genders, out of which female pups exhibited more dynamism than the male counterparts [42]. PPA administration to rats significantly and consistently evoked abnormal behavioral patterns similar to ASD patients. However, PPA administration does not affect routine motor functions such as distance traveled, swim speed, walking ability, etc [16]. Nevertheless, fine motor skills such as vehicle driving, shaving, and dancing are found to be impaired in ASD patients.

• Impact of PPA on EEG and Brain Function

A high incidence of epileptic seizures occurs along with other behavioral abnormalities in ASD children. ICV chronic administration of PPA results in the development of limbic-type kindled seizures [15]. Furthermore, it was found that low (4 µl of 0.052 M solution) and high (4 µl of 0.26 M solution) doses of PPA administered ICV to rats once a day for 5 consecutive days produced progressive EEG spiking and accompanying convulsive activity [15]. This epileptiform activity typically appeared first in the hippocampus, followed seconds later by the neocortex and caudate activity. Epileptiform movement during initial sessions was usually accompanied by either no convulsive behavior or Stage 1 convulsions. However, during subsequent sessions, epileptiform activity strengthened in all three sites, and the severity of convulsions progressed, culminating in Stage 4 or 5 convulsions in rats given the high dose of PPA. Thus, these findings of PPA-induced seizures and abnormal EEG activity are consistent with increased seizure and movement disorder in ASD [15, 16, 29].

Mechanism of Action of PPA

Propionic acid (PPA) is capable of crossing the gut- the blood, blood-brain, and placental barrier, exerting diverse effects on various endogenous processes. PPA significantly alters intracellular pH, producing acidosis. PPA interferes with cell signaling neurotransmitter activity and induces closure of synaptic gap junctions. Furthermore, PPA impairs mitochondrial function and lipid metabolism. PPA not only produces neuroinflammation but also interferes with gene expression [15, 16].

Intracellular Acidification by PPA

It is a common observation that humans suffering from metabolic acidosis often experience confusion, agitation, and other behavioral abnormalities similar to symptoms observed in ASD [43]. The carboxylate functional group and/or acidic properties of PPA induce intracellular acidification in brain cells when it gains access to the CNS by either passive or active transport, owing to its small molecular weight. The consequences of reduced intracellular pH by PPA on cellular physiology are complex and produce diverse untoward effects such as social deficits and abnormal behavior [16]. Carnitine plays a critical role in energy production. It is an essential co-factor that helps transport long-chain fatty acids into the mitochondria so that they can be oxidized to produce energy in the form of ATP. PPA-induced intracellular acidification inhibits carnitine function, which is vital for the normal metabolism of long-chain fatty acids [44]. PPA sequesters carnitine function and inhibits coenzyme A activity, impairing mitochondrial metabolism and energy production. Thus, by inhibiting carnitine function, PPA can further decrease cytoplasmic pH by accumulating long-chain fatty acids [44]. There is evidence of impaired fatty acid metabolism in ASD patients, including carnitine deficiency, mitochondrial dysfunction, and systemic elevations of nitric oxide metabolites [20, 45]. An impaired fatty acid metabolic process leads to various negative effects on the CNS, resulting in increased oxidative stress. Organic acidemias produce abnormal behavioral symptoms, as observed in PPA animal models and ASD patients [27]. PPA ICV administration increases oxidative stress markers as well as abnormalities in glutathione-associated pathways [15]. Glutathione participates in both antioxidant defense and xenobiotic detoxification over a variety of environmental organic compounds and metals [46]. The glutathione system consists of both primary

and secondary antioxidants, including glutathione peroxidase, glutathione reductase, glutathione S-transferase, g-glutamyl cysteine synthetase, and glucose-6-phosphate dehydrogenase [15]. Alterations in the activities of these enzymes suggest reduced cellular defense and are considered markers of increased oxidative stress. ASD patients show impaired glutathione-associated pathways [46]. Along similar lines, ICV-PPA-induced metabolic acidosis also increases oxidative stress, as reflected by impaired glutathione pathway [16].

Inhibition of Gap Junction-Dependent Intercellular Communication by PPA

An intact synaptic gap is essential for neurotransmission and normal cell-to-cell communication. During the early years of brain development, gap junction coupling is vital for synchronizing neural electrical activity and transmission of neuronal signals. ICV administration of PPA produces closure of these gap junctions, thereby impairing neuronal signals and resulting in stereotypical movements, hyper locomotion, and disruption of motor function in rodents [15-16, 29].

Elevation of Neurotransmitter levels by PPA

It has been reported that ASD patients have elevated serotonin and dopamine levels. Furthermore, medications targeting the serotonergic and dopaminergic systems reduced the severity of ASD symptoms [28, 47, 48]. Interestingly, PPA can enhance both dopamine and serotonin levels in rats in a manner similar to what is observed in ASD patients. Since PPA can rapidly enter brain cells, it accumulates intracellularly and increases serotonin and dopamine levels via reducing intracellular pH and potentiating intracellular calcium release [28, 47]. Additionally, PPA increases the synthesis of dopamine and related catecholamines through the induction of tyrosine hydroxylase, a key enzyme in the synthesis of catecholamines. These elevated serotonin and dopamine levels are responsible for abnormal social and motor behaviors [28, 47, 48]. Glutamate is the crucial excitatory neurotransmitter involved during the child's brain development. Glutamate has a big role in neuronal proliferation, migration, synapse maturation, and cell differentiation.

On the other hand, GABA is an inhibitory neurotransmitter that balances the actions of glutamate. Several reports in the literature point out the imbalance between glutamate and GABA, particularly in ASD patients. The plasma GABA and glutamate concentrations are altered in autistic children. Propionic acid displays similar alterations in the levels of glutamate and GABA in rodents [21]. Acetylcholine (Ach) is the principal neurotransmitter that plays a major role in learning and memory processes [49]. PPA, when administered ICV, interferes with the normal synthesis of Ach and the activity of Ach at the synapse, which perhaps explains cognitive decline observed in both adult rodents administered with PPA and ASD children [21]. Abnormalities in the dopaminergic, serotonergic, GABAergic, and cholinergic systems appear to be implicated in cognitive, behavioral, motor, and social deficits in both humans and rats [21, 28, 47-50]. To summarize, alterations in the levels of certain neurotransmitters such as catecholamines, serotonin, acetylcholine, GABA, and glutamate induced by PPA plausibly explain the behavioral abnormalities observed in both the PPA animal model and ASD patients [16, 21, 28, 47].

PPA Interferes with Mitochondrial Function

The crucial constituents of neuronal membranes are comprised of polyunsaturated fatty acids (PUFAs). PUFAs play a key role in the efficient functioning of the brain and its development [51]. The physiological activity of neuronal membrane proteins depends largely on phospholipid content, which can be affected by environmental factors implicated in ASD [52]. When administered intracerebroventricularly for seven consecutive days (4 µl of 0.26 M solution), PPA evoked hyperactivity and abnormal behavior in rats [31]. It was observed that the lipid profile of rat brain phospholipids was significantly altered upon PPA treatment, indicating a close link between ASD symptoms, lipid profile, and PPA [31]. The transport of fatty acid across the mitochondrial membrane for energy production cannot occur without carnitine. There is sufficient evidence pointing towards lowering carnitine levels and accumulation of acylcarnitine's (inactive form) in mitochondria after chronic administration of PPA, thereby disturbing mitochondrial premiere function of energy production. This finding is consistent with the abnormal lipid profile of ASD patients and is further substantiated by the fact that carnitine supplementation improves ASD symptoms [20]. The underlying mechanism of PPA-producing ASD symptoms appears to be related to its binding ability to propionyl coenzyme A and sequestering carnitine, thereby interfering with mitochondrial fatty acid metabolism [53]. These findings suggest Autism is a disorder of impaired mitochondrial function, at least to some extent in genetically susceptible individuals [16].

PPA Causes Neuroinflammation

Several investigators have consistently found that ICV injections of PPA in rats evoke a neuroinflammatory response characterized by increased activated microglia and reactive astrogliosis in brain areas, including the hippocampus, neocortex, and white matter [15]. Propionic acid (PPA), when administered at 250 mg/kg/day orally for 3 days, also produced significant neuroinflammation in rats [54]. In support of this hypothesis, neuroinflammatory responses have been found in several neurological disorders, including Autism, and are thought to contribute to symptomatology. The hippocampus and neocortex are the brain areas that primarily maintain social skills, cognitive function, and normal behavioral patterns [28, 47]. Administration of PPA produces neuroinflammation in these brain structures and impairs social skills, evoking stereotypic behavior and cognitive decline, the core symptoms of ASD. Post-mortem studies on the brains of ASD patients revealed increased activated microglia and reactive astrocytes in the hippocampus, neocortex, and white matter suggesting that an inflammatory process may be present throughout the life span of ASD individuals [55]. Taken together, the neuroinflammatory findings from the PPA model appear consistent with the clinical situation of ASD [16, 28, 47, 54].

Alteration of Gene Expression by PPA

The cyclic AMP-responsive element-binding protein (CREB) is a ubiquitously expressed nuclear transcription factor that gets activated by various extracellular stimuli. CREB is known to regulate the expression of genes essential for cell survival, proliferation, adaptation, and differentiation. PPA modulates histone acetylation, thereby inducing phosphorylation of CREB. Thus, this important neuroregulatory protein plays a key role in the physiological expression of genes necessary for maintaining neuroplasticity, normal movement, mood, and memory. PPA

alters this adaptive gene expression required for cell survival, thereby interfering with cell physiology resulting in stereotypic, repetitive behaviors, cognitive decline, and memory impairment [15-16, 29]. When administered subcutaneously at a dose of 500 mg/kg/day for five consecutive days, PPA significantly enhanced the expression of the glial fibrillary acidic protein (GFAP), an astrocyte-related gene. In contrast, the expression of octamer-binding transcription factor 4 (OCT4), a neural stem cell-related gene, was significantly decreased. In addition, PPA-treated rats showed significantly increased expression of the pro-inflammatory gene TNF-α [35].

Concluding Remarks

Autism spectrum disorder is a multi-factorial, neurodevelopmental disability manifesting its symptoms in childhood with a dynamic set of metabolic, mitochondrial, immune, neuroinflammatory, and behavioral abnormalities affecting several body parts. Appropriate animal models that can resemble core symptoms of Autism, such as defective social skills, ritualistic behavior, and cognitive decline, are difficult to develop. Nevertheless, it becomes crucial to evoke these bizarre behavioral signs in small laboratory animals to explore the pathogenesis of ASD and discover potential medicines useful for this disorder. No promising biomarkers have been identified to date, which can give clues about the likelihood of ASD. Propionic acid (PPA), being available endogenously as an intermediary product of cellular fatty acid metabolism and end product produced by the intestinal tract, skin, and oral mucosa bacteria, finds its way to reach the female uterine environment in the human setting.

Furthermore, in addition to its endogenous synthesis, PPA is a popular preservative in various foodstuffs. Although PPA may be beneficial at optimum levels, excessive PPA appears to have several undesirable effects on health. PPA can readily cross the gut-blood, blood-brain, and placental barriers. PPA particularly gets concentrated intracellularly, producing deleterious effects on the developing brain. This becomes noteworthy in the context of ASD since PPA affects cell signaling, neurotransmitter synthesis, mitochondrial function, lipid metabolism, immune function, neuroinflammation, and gene expression, all of which have been implicated in ASD. There is ample evidence suggesting that genetic susceptibility, adverse environment, and uterine factors interplay with one another in a complicated manner, consequently yielding an autistic child. Animal models serve as useful tools in preclinical research to test the potential of new medicines. The PPA rodent model of autism shares many common features with autistic children, such as altered cellular neurochemistry, abnormal behavioral patterns, impaired lipid metabolism, and cognitive decline. The observations with acute and chronic administration of PPA in rodents suggest that PPA could be the culprit responsible for the behavioral, neuropathological, and biochemical abnormalities observed in ASD. PPA could be the intrauterine candidate accountable for causing harm to the unborn child, resulting in the development of an autism-like disorder. The PPA model exhibits excellent construct and face validity. A thorough analysis of the data available in the literature from preclinical studies as well as the clinical reports justifies the utility of the PPA rodent model of Autism as a tool in neurochemistry and experimental pharmacology.

Acknowledgement

The first author, Priya Joon is (DST, Govt. of India, New Delhi) Inspire Research Scholar

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