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ORIGINAL ARTICLE

Serum Galanin in Children with Autism Spectrum Disorder

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Abstract

Recent studies have attempted to measure several biomarkers to understand the complex interactions of the anatomic systems that may be involved in autism spectrum disorder (ASD). In CNS, galanin takes part in a variety of pathological and physiological processes. Prior research has indicated it is involved in several neuropsychiatric disorders and has a role in inhibiting the neuronal firing and release of serotonin, norepinephrine, and acetylcholine. To date, serum galanin levels have not been investigated in the context of ASD. This study aimed, therefore, to compare the serum galanin levels of children with ASD and healthy controls and to reveal any association between galanin level and the severity of ASD, as well as other psychological and demographic parameters. Serum galanin levels were measured by radioimmunoassay in 116 children with ASD and 98 healthy children. We observed significantly increased serum concentrations of galanin in children with ASD relative to healthy children. Moreover, children with severe ASD had significantly higher galanin levels than those with less severe disease. We also confirmed significant positive correlations between galanin and psychiatric parameters in children with ASD. For the first time, we suggest a possible correlation between serum galanin and the degree of ASD severity. Increased galanin levels may play a role in the pathogenesis of ASD.

Keywords Autism · CARS · Children · Galanin

Introduction

Autism spectrum disorder (ASD) is a heterogeneous group of neuropsychological disorders characterized by disruptions in social, communicative, and cognitive development, together with repetitive and restricted behavioral patterns and interests. ASD is usually diagnosed before the age of 3 years [1, 2]. No known causes have been identified to explain the significant increase in the prevalence of ASD in the last decade [3]. However, although the exact etiology of ASD is not entirely understood, some researchers believe a genetic predisposition may exist and that environmental and immunological factors may be involved in the pathogenesis of this condition [3]. Galanin is a 30–amino acid neuropeptide (29–amino acid neuropeptide in animals) widely distributed in the neurons of both the central (CNS) and peripheral nervous systems. Three galanin receptor types (GAL1–3) have been cloned to date. GAL1 and GAL2 receptors are found mainly in the CNS [4] and predominantly activate inhibitory G proteins, while GAL2 receptors mediate excitatory signaling [4, 5]. Generally, galanin receptors are

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involved in many neuroendocrine, addiction, pain, cardiac, and metabolic functions and also play an essential role in glucose homeostasis in humans and experimental animals [4–7]. In the CNS, galanin takes part in a variety of pathological and physiological processes. Prior research has indicated it is involved in several neuropsychiatric disorders, e.g., major depression disorders, and has a role in inhibiting the neuronal firing and release of serotonin, norepinephrine, and acetylcholine. Also, galanin was found to be involved in the regulation of mood, including anxiety and depression, in animal models [6, 7]. Based on experimental and human studies, galanin has been proposed to regulate several CNS physiologic actions [4, 9, 10]. Experiments using receptor-selective agonists and antagonists and various animal models have indicated that galanin receptors are principally involved in several neuropsychological actions [7, 9, 10]. Considering the substantial number of established and putative galanin signaling physiologic activities, galanin has been correlated with metabolic regulation, osmotic homeostasis, reproduction, nociception, arousal/sleep, and cognition [5–10]. These functions are linked to the actions of GAL1 and GAL2 receptors [7–10]. GAL1 receptors have been linked to the CNS and peripheral nervous system and neurotransmission modulatory activity in anxiety and nociception, whereas GAL2 receptors are widely expressed in the CNS and involved in neurodevelopment; the modulation of affective behaviors; and neuronal outgrowth, survival, and hippocampus neurogenesis [9–11]. Besides, galanin and its receptors have been linked to the neurogenesis of adult and embryonic neural stem cells [11]. Galanin receptors mainly mediate the effects on serotonin release in vivo in the dorsal raphe [9]. Furthermore, galanin modulates serotonin receptors (i.e., 5-HT1A) at postsynaptic and autoreceptor levels in the CNS [8, 9].

Recent studies have attempted to measure several biomarkers to understand the complex interactions of the anatomic systems that may be involved in ASD. Both these previous studies and our work might help to elucidate potential ASD etiologic pathways, differentiate individuals with ASD from those without, and establish subgroups of cases that may be phenotypically similar. Further research that builds upon the available literature could help to establish new drugs targeting these biomarkers or their receptors as a novel treatment for ASD. To date, serum galanin level as a new biomarker has not been investigated in children with ASD. Thus, our study aimed to compare the serum galanin levels of children with ASD and healthy controls and to reveal any association between the level of galanin and the severity of ASD and other psychological and demographic parameters.

Methods

Study Participants

This case–control study was undertaken at Assiut University Hospital, Egypt, conducted following the code of ethics of the World Medical Association (Declaration of Helsinki) for human experiments, and was approved by Assiut University's Hospital Ethical Scientific Committee. The caregivers of all study participants gave their informed written consent prior to study enrollment. The study was conducted from June 2018 to May 2019 (no. 13-3/2019).

Study participants were required to have a diagnosis of ASD established according to the Diagnostic and Statistical Manual of Mental Disorders, fifth edition [12] to be eligible for inclusion in this study, while the presence of any of the following diseases or conditions led to exclusion: (i) chronic renal, endocrine, cardiac, and hepatic disorders; (ii) metabolic disorders, e.g., dyslipidemias, pyruvate carboxylase deficiency, or porphyria; (iii) failure to thrive. A total of 116 children from 115 families, selected from among 137 children with ASD, were finally included in this study. Of the 21 children excluded from this investigation, 17 did not meet the inclusion criteria and/or have one or more of the exclusion criteria, and four families declined to allow their child to participate in the study. All ASD participants were recruited from Assiut University Hospitals' neuropediatric clinics and two private centers in upper Egypt and were receiving behavioral therapy only without medications 3 months before the study. Separately, the study included 98 age- and sex-matched healthy children as controls. All controls were free from any psychiatric disorders, and the conditions or diseases listed as reasons for exclusion.

Procedures

Detailed medical history-taking and physical examinations were conducted for all participants with ASD, including the collection of a family history of consanguinity, any psychiatric diseases in the family, social activities, and the time of ASD diagnosis. Anthropometric measurements were completed for all participants with ASD and healthy controls. A senior psychiatrist established the diagnosis of ASD in each patient before their recruitment into the study. The determination of ASD diagnosis was made using the Diagnostic and Statistical Manual of Mental Disorders, fifth edition [12], and the Autism Diagnostic Interview—Revised (ADI-R) tool [13]. Two parent interviews were organized, with the first session reserved for ASD diagnosis and the other session conducted to evaluate

ASD severity and confirm the diagnosis using the Childhood Autism Rating Scale (CARS). The CARS assesses the behavior in 14 domains of ASD and one parameter of autism impression [14].

Blood samples (5 mL of blood per participant taken from a vein in the antecubital fossa without venous occlusion) for analyzing the galanin level were collected from the study participants following an overnight fasting period lasting 10 to 12 h. We used a serum separator tube and allowed the samples to clot, then centrifuged each at 3000 rpm for 10 min. The serum samples were collected into Eppendorf tubes with plastic Pasteur pipettes, then stored at − 80 °C until later biochemical investigations. Galanin level was assayed by an enzyme-linked immunosorbent assay kit for in vitro quantitative measurement of the galanin level in human serum (catalog no. CEB084Hu; USCN Life Science Inc., Wuhan, China). This assay employed the competitive inhibition enzyme immunoassay technique: first, a monoclonal antibody specific to galanin was precoated onto a microplate. A competitive inhibition reaction was established between biotin-labeled galanin and unlabeled galanin with the precoated galanin antibody, with any unbound conjugate subsequently washed off after incubation. Next, avidin conjugated with horseradish peroxidase was added to each microplate and incubated. The amount of conjugate was reversely proportional to the galanin concentration in the sample. Following the addition of a substrate solution, the intensity of the color that developed was considered as reversely proportional to the galanin concentration. The detection range was 12.35 to 1000 pg/mL; the minimum detectable dose of galanin was less than 5.25 pg/mL. Intra-assay coefficients of variability were less than 10%. All biochemistry measurements were assayed at Assiut University Hospital's clinical laboratory.

Statistical Analyses

We used the Statistical Package for the Social Science version 22 software program (IBM Corporation, Armonk, NY, USA) for data collection, revision, and coding. Normally distributed data were calculated as mean ± standard deviation (SD) values, and non-normally distributed data were calculated as median and interquartile range values. Significant differences between participants with ASD and healthy controls were calculated using a t-test for normally distributed data and the Student's Wilcoxon signed-rank test for non-normally distributed data. Pearson's or Spearman's correlation coefficient tests were used for the univariate analysis of the correlation between galanin level and psychiatric, anthropometric, and biochemical parameters. A p-value of less than 0.05 was considered to be statistically significant.

Results

Table 1 presents study participant (n = 116) demographics, including age, sex, body mass index, age at the diagnosis of ASD, CARS score, and ADI-R score. There were no significant differences between the participants with ASD and healthy controls in terms of age, sex, and body mass index. About two-thirds of ASD children were diagnosed before the age of 3 years. CARS scores ranged from 30.5 to 53.7 points (mean: 36.2 ± 5.9 points), with 56% of ASD group having mild/moderate ASD and 44% having severe ASD, respectively. Table 2 shows the serum galanin levels in children with ASD as compared with those in age-matched controls. We found that a significantly increased serum concentration of galanin existed in children with ASD when compared with the control group ($p < 0.001$). Moreover, children with severe ASD had significantly higher galanin levels than those with less severe disease ($p < 0.01$). Additionally, we observed no significant differences in galanin level according to sex or age among children with ASD (Table 2). Table 3 shows the correlation analysis between the serum galanin level and other parameters in children with ASD. In the univariate analysis of data, galanin level was significantly positively correlated with the ADI-R parameters "communication" ($r = 0.298$; $p < 0.01$) and "social interaction" ($r = 0.452$; $p < 0.05$) and the CARS parameters "relating to people" ($r = 0.694$; $p < 0.05$), "adaptation to change" ($r = 0.306$; $p < 0.05$), "verbal communication" ($r = 0.271$; $p < 0.01$), "nonverbal communication" ($r = 0.528$; $p < 0.05$), "fear" ($r = 0.525$; $p < 0.05$), and "activity level" ($r = 0.448$; $p < 0.05$) as well as the total CARS score ($r = 0.631$; $p < 0.05$) (Table 3).

Discussion

It is currently accepted that ASD is a developmental disorder resulting from the interruption of typical neurobiological mechanisms; however, the exact processes and molecules that may be involved in ASD are still under investigation [3, 15, 16]. To our knowledge, our study was the first to evaluate serum galanin levels in a cohort of children with ASD. Our results confirmed both that significantly higher serum galanin levels existed in children with ASD than in healthy children and that these levels were higher in those with severe ASD than in those with mild to moderate ASD, suggesting that galanin might play a role in ASD pathogenesis. Galanin is expressed with numerous neurotransmitters and neuropeptides in various types of CNS neurons. Disturbances in the galaninergic system have been identified in many neuropsychiatric

Table 1 Anthropometric, demographic and autism data of all study subjects

Variable	ASD children (N = 116)	Control (N = 98)	p-value
Age, years (mean ± SD)			
Range	3.5–11	3–12	0.67
Mean ± SD	4.25 ± 3.45	4.5 ± 2.9	
Gender [number (%)]			
Males	92 (79.3%)	79 (80.6)	0.97
Females	24 (20.7%)	19 (19.4)	
BMI, Kg/m^2 (mean ± SD)	22.18 (± 3.24)	21.83 (± 3.67)	0.31
Age at diagnosis [number (%)]			
Before 3 years	78 (67.25%)	–	–
After 3 years	38 (32.75%)	–	–
Childhood Autism Rating Scale (CARS)			
Range	30.5 to 53.5	–	–
Mean ± SD	36.2 ± 5.9	–	–
Mild/moderate (≤ 36.5) number (%)	65 (56%)	–	–
Severe (≥ 37) number (%)	51 (44%)	–	–
Autism Diagnostic Interview-Revised" (ADI-R)			
ADI-R Communication	18.2 ± 6.2	–	–
ADI-R Stereotyped behavior	3.5 ± 1.7	–	–
ADI-R Social interaction	12.4 ± 5.3	–	–
ADI-R Play	5.8 ± 2.1	–	–

Table 2 Serum Galanin in autistic and control groups, different CARS and age groups in autistic patients

Parameter	Group (number)	Mean ± SD	*p*-value
Serum Galanin (pg/mL)	Autism (116)	59.05 ± 8.11	< 0.001*
	Control (98)	27.47 ± 10.70	
Age of autism group	Below 6 years (67)	58.39 ± 7.33	0.682
	Above 6 years (49)	58.09 ± 7.08	
CARS grades of severity	Mild/moderate (≤ 36.5) (65)	48.7 ± 9.80	< .01*
	Severe (≥ 37) (51)	61.85 ± 7.18	
Gender of autism group	Male (92)	57.66 ± 6.76	0.082
	Female (24)	58.33 ± 7.43	

*Significant

human diseases such as seizures, epilepsy, anxiety disorders, major depression, Alzheimer's disease, chronic pain, and substance abuse [6, 7, 17]. Galanin receptors are widely expressed in various tissues, especially the CNS. GAL1 is mainly found in the olfactory system, amygdala, hypothalamus, thalamus, and brain stem. Meanwhile, GAL2 messenger RNA is expressed in the hippocampus, trigeminal tract, gyrus dentatus, and dorsal vagal complex, while GAL3 is found only in the hypothalamus [17, 18]. The participation of galanin in psychiatric disorders is a crucial point requiring clarification. Previous studies have reported that galanin has an anxiolytic effect when administered in the CNS of experimental animals (see 17 for more details). Swanson et al. [19] reported that selective GAL3 antagonists have antidepressant and anxiolytic effects in animals, which is attributed to a decrease in galanin's inhibitory effect on serotonin transmission at the dorsal raphe nucleus.

We confirmed significant positive correlations between the serum galanin level and certain behavioral and psychological parameters in patients with ASD, including communication ($p < 0.01$), social interaction ($p < 0.05$), relating to people ($p < 0.05$), adaptation to change ($p < 0.05$), verbal ($p < 0.01$), nonverbal communication ($p < 0.05$), fear ($p < 0.001$), and activity level ($p < 0.001$) (Table 3). To date, research has not definitively revealed the causes of autism-related cognitive impairment, even in children with apparent chromosomal aberrations [20]; however, our current findings

Table 3 Correlation between Galanin levels and psychiatric parameters

Serum galanin	Univariate analysis	
	r	p-value
ADI-R parameters		
ADI-R communication	0.298	< 0.01*
ADI-R STEREOTYPED behavior	0.275	0.173
ADI-R Social interaction	0.452	< 0.05*
ADI-R play	0.199	0.134
CARS parameters		
Relating to people	0.694	< 0.05*
Emotional response	0.198	0.103
Imitation	0.215	0.672
Body use	0.231	0.169
Object use	0.189	0.127
Adaptation to change	0.306	< 0.05*
Listening response	0.107	0.029
Taste, smell, touch	0.116	0.203
Visual response	0.134	0.311
Fear	0.525	< 0.05*
Verbal communication	0.271	< 0.01*
Activity level	0.448	< 0.05*
Non-verbal communication	0.528	< 0.05*
Level and consistency of intellectual response	0.154	0.101
General impression	0.164	0.106
Total CARS scores	0.631	< 0.05*

*Significant

suggest the possibility that galanin, as a neuropeptide, may have a role in the pathogenesis of ASD behavior. Aberrant galanin expression in the CNS was observed in several pathological conditions, suggesting a role for the neuropeptide/receptor system in the development, pathology, or response to neuronal damage and neurodegeneration [6, 7, 10, 11]. Galanin's role in memory, learning performance, and behavior has been previously reported in the literature [4, 9, 17]. Galanin administration hampered memory-formation processes and impaired learning performance in transgenic galanin-overexpressing mice. Moreover, some reports have highlighted the existence of the same deficits in emotional memories and learning with significant effects on olfactory and spatial navigation tests [9, 17, 21, 22]. Galanin's inhibitory properties in the CNS appear mainly via potassium hyperpolarization, accompanied by diminished input resistance with a reduction in presynaptic excitatory inputs [23].

In animal model research studies, endogenous and exogenous galanin was found to modulate depressive- and anxiety-like behaviors [8]. Treatment with galanin or its agonists in animal models of depression affected or changed rodent behaviors and the response to and neurochemical actions of antidepressant drugs. Conversely, antidepressant therapy affects both galanin and galanin-receptor gene expression in rodents [4, 24]. The exact pathophysiology of ASD remains unclear but is thought to include disturbances in monoaminergic brain transmission [25]. Previous studies have found alterations in galanin and/or galanin receptors to be correlated with anxiety disorders and major depression [4, 6, 26]. Wang et al. [6] investigated galanin levels in 79 patients with major depression and reported that significantly higher plasma galanin levels existed in those with depression than in patients in remission and healthy controls ($p < 0.05$). Furthermore, these researchers also confirmed a positive correlation existed between galanin and severe depression in female patients ($p = 0.020$) [6]. Galanin is coexpressed with and modulates serotonin and norepinephrine transmissions, which are involved in depression and other psychiatric diseases. Elsewhere, Juhasz et al. [26] reported the roles of galanin and galanin receptors in the pathology and potential treatment of major depressive disorder. Other studies have also focused on the nature of galanin signaling in the central amygdala, which is a biogenic site for anxiety-like behavior production. In one study, galanin had dual effects on γ-aminobutyric acid (GABA) transmission, decreasing the amplitudes of GABAergic inhibitory postsynaptic potentials in most of the central amygdala neurons and increasing inhibitory postsynaptic potentials in others [27]. Our work presented here can be considered the first step in the process of elucidating the potential role of galanin in ASD. We suggest that future research focus on the actions of galanin and galanin receptors in a manner that could help to establish new drugs targeting these biomarkers or their receptors.

Limitations and Strengths

This study is preliminary in nature (containing a relatively small sample size and being potentially the first study of its kind), and the reported data are correlative and gathered from children ages 3 to 12 years. We suggest that future investigations into the area of the actions of galanin and galanin receptors in ASD be conducted.

However, we found a possible correlation between serum galanin and the degree of severity of ASD for the first time as the observed increased galanin levels suggested that galanin may have a role in the pathogenesis of ASD. Galanin could be a valuable biomarker for ASD, which could assist in future research on the pathogenesis and possible ASD interventions.

Summary

Galanin is coexpressed with and modulates serotonin and norepinephrine transmissions, both of which are implicated in depression and other psychiatric diseases. Significantly

higher concentrations of serum galanin were found in children with ASD as compared with among healthy children. Moreover, children with severe ASD had significantly higher galanin levels than those with milder disease. We also observed that significantly positive correlations existed between galanin and some psychiatric parameters in children with ASD. Galanin could be a valuable biomarker for ASD, which could support future research on the pathogenesis and the development of possible interventions in ASD.

Authors Contributions KS, Ah AA and KHM conceptualized and designed the study protocol development, assessment and writing the manuscript. Ah AA and Ab AA Performed All neuropsychiatric assessments of all cases and reviewed and revised the manuscript. AMA, AE, ARA, MME designed the data collection instruments, and coordinated and supervised data collection and statistical analysis. EMN, OE, and DAN performed laboratory analysis. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Compliance with Ethical Standards

Conflict of interest All authors do not have a potential conflict of interest.

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