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Plenary article

The relationship between the neuromodulator adenosine and behavioral symptoms of autism

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ABSTRACT

The neuromodulator adenosine is an endogenous sleep promoter, neuroprotector and anticonvulsant, and people with autism often suffer from sleep disruption and/or seizures. We hypothesized that increasing adenosine can decrease behavioral symptoms of autism spectrum disorders, and, based on published research, specific physiological stimuli are expected to increase brain adenosine. To test the relationship between adenosine and autism, we developed a customized parent-based questionnaire to assess child participation in activities expected to influence adenosine and quantify behavioral changes following these experiences. Parents were naive to study hypotheses and all conditions were pre-assigned. Results demonstrate significantly better behavior associated with events pre-established as predicted to increase rather than decrease or have no influence on adenosine. Understanding the physiological relationship between adenosine and autism could open new therapeutic strategies – potentially preventing seizures, improving sleep, and reducing social and behavioral dysfunction.

Autism spectrum disorders (ASDs) affect as many as 1 in 110 individuals, and educational and developmental interventions demand significant public and private resources. Genes play a role [50], but, in the majority of cases, the underlying genetics are complex and involve at least 10 different genes [35], most likely interact with environmental factors [23], and are unlikely to be addressed via gene therapy in the foreseeable future. Early detection offers the potential to improve long-term outcomes, and in some cases genetic disorders – e.g., metabolic disorders such as phenylketonuria (PKU) – can be managed symptomatically with excellent results.

Neurotransmitters commonly linked to ASD include serotonin, dopamine, GABA, glutamate and acetylcholine [39], and interventions include antidepressant, antianxiety, antipsychotic and antiepileptic drugs [38]. Typically drugs target symptoms (e.g., inattention or internalizing disorders) rather than core impairments, often with significant side effects. New and better approaches are needed.

Here we explored the relationship between adenosine, a sleep-promoting, seizure-reducing neuromodulator [10], and behavioral symptoms of ASD (e.g., poor eye contact, repetitive movements). ASDs are associated with increased incidence of epilepsy and sleep disorders [30] – serious comorbidities in terms of medical management and quality of life. Adenosine is the core molecule of adenosine triphosphate (ATP), and decreased activation of the widely distributed adenosine A_1 receptor (A_1R) increases anxiety and seizure susceptibility in rodents; conversely, A_1R activation has well-established anticonvulsant and neuroprotective properties [10]. Adenosine A_{2A} receptor ($A_{2A}R$) expression is low in most areas, but high in dopamine-containing regions such as the basal ganglia; $A_{2A}R$ activation has been associated with reduced perseverative behaviors [45]. Caffeine, the most widely used psychoactive drug, is an adenosine receptor antagonist with potential for multiple neurological and psychiatric disorders [27,41]. Based on behavioral and physiological characteristics of ASD, including impaired sleep, increased seizures [5,30] and perseverative behaviors [29], an insufficient influence of adenosine may underlie some symptoms. Alternatively, even if levels are normal, increased adenosine may still be beneficial. We predict it could offer short term improve-

Abbreviations: ASD, autism spectrum disorders; A_1R, adenosine A_1 receptor; $A_{2A}R$, adenosine A_{2A} receptors.

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ments [33] with the potential to facilitate more long-term changes (e.g., decreased motor stereotypies might facilitate pro-social interactions).

It is impractical to measure CNS adenosine in humans, and peripheral adenosine levels (e.g., in plasma) are not informative. Nevertheless, basic and translational research has demonstrated that mechanical pressure or sudden physical impact [18], seizures [48], intense exercise [12], increased temperature (by 2 °C) [32], decreased pH (by 0.3 units) [8,9] and reduced glucose [26] all serve to increase brain adenosine directly or indirectly (via ATP dephosphorylation) within minutes, and lasting up to hours. Recent publications suggest that a ketogenic diet, an effective treatment for pediatric epilepsy and reported to improve autism [15] can act via A_1Rs [34].

In parallel, many repetitive and self-stimulatory activities characteristic of autism would be predicted to release ATP and/or adenosine. For example, rocking, spinning and Grandin's "hug machine" [13] exert mechanical pressure or induce sudden changes in acceleration, and thus may exert their effects through increased extracellular adenosine, similar to local increases via impact or acupuncture [19,21]. Conversely, basic research indicates that hyperglycemia and increased pH each could decrease adenosine acutely [8,9,40]. Because adenosine is uniquely poised to link metabolism and brain activity, we speculate that some behaviors which increase adenosine could represent metabolic reinforcement – a form of self-medication by attempting to self-regulate neuronal excitability through increased adenosine. Engaging in adenosine-increasing activities could thus lead immediately to decreased symptoms of autism.

To explore the possibility of a beneficial relationship between autism and adenosine – as suggested by animal models – we administered a customized behavioral questionnaire to parents of children with a confirmed ASD diagnosis via a validated national autism database. Questions were designed to examine behavioral changes following activities identified (based on research described above) as expected to increase, decrease or have no effect on adenosine. The present report reveals a significant relationship between engaging in stimuli preassigned as expected to increase adenosine and parent report of decreased severity of ASD symptoms.

Subjects were recruited with the assistance of the Interactive Autism Network (IAN) Research Database at the Kennedy Krieger Institute and Johns Hopkins Medicine – Baltimore, sponsored by the Autism Speaks Foundation. Autism diagnostic status was confirmed by IAN staff; prior work has shown that fully 98% of participants ascertained as on the spectrum according to IAN standard phenotyping procedures were ASD-positive according to clinicians' best estimate [28]. Participants had received a formal autism diagnosis according to DSM-IV criteria made by a qualified professional (e.g., psychologist, psychiatrist) and were above a threshold score of 12 on the Social Communication Questionnaire (SCQ) parent-report measure [42].

2000 parents of a child with a diagnosed ASD were invited to complete an internet-based questionnaire. 201 parents began and 191 (83%) completed it; after excluding participants over age 18, there were data for 166 children. Overall responses on 166 completed questionnaires contained information sufficient to generate scores for adenosine-increasing or non-increasing activities. Comparisons of available data indicated no significant group differences between those who did and did not complete it.

Enrollees ranged from 2.4 to 18 years, with a mean age of 9.5 years (SD = 3.8); there was a typical gender distribution of 138 (83%) males and 28 (17%) females. The autism diagnoses as identified by parents included Autistic Disorder ($n = 101$), Asperger Syndrome ($n = 32$), and Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS; $n = 33$). Parents described their child's verbal level as nonverbal ($n = 18$), using single words ($n = 12$), using phrases ($n = 46$), or fully fluent ($n = 84$).

The customized questionnaire included categories with analogous adenosine-increasing or -neutral/decreasing conditions based on published basic and translational research; examples of probed activities are listed in Table 1. Adenosine-stimulating activities included high-intensity physical conditions, raising body temperature, and adhering to a low-carbohydrate (ketogenic) diet. Adenosine-neutral or -decreasing activities included consuming foods likely to lead to a state of hyperglycemia, gentle exercise, breaking a bone, or watching television. Caffeine is an adenosine receptor antagonist, thus blocking partially the effects of endogenous adenosine; chronic caffeine exposure upregulates receptor levels. Control activities were chosen to be similarly engaging and/or pleasurable as adenosine-stimulating activities.

After an episode of participation (10 min minimum, for intense (exhaustive) activity like the trampoline), parents reported whether they observed changes in functioning (within 1 h; 12 h for medical events; not specified for ongoing diet). Overall, categories were based on adenosine research with rodent models, coupled where possible with an established metabolic equivalent (MET; defined as work metabolic rate/basal metabolic rate) in humans [1]. Adenosine levels can change within minutes, and relevant timelines/intensities were established to avoid borderline changes with criteria for physical activities of >6.0 MET for adenosine-increasing activities and <3.0 MET for adenosine-decreasing or neutral activities. Ideally we would test the impact of behavioral activities on adenosine regulation in humans; however direct physiological assessments of adenosine are not feasible – blood and urine do not reflect central adenosine, and central nervous system measurements or manipulations are overly invasive at this point. Thus, data from this parent-report questionnaire provide an initial indirect and non-invasive test of this novel hypothesis.

Using a 5-point Likert scale ("lots of decline," "some decline," "no noticeable change," "some improvement," "lots of improvement"), parents reported whether they saw changes in each of nine domains (Table 2): social and communicative skills; repetitive or perseverative activities; eye contact; social interest; sound sensitivity; sleep; anxiety; adapting to transitions; and aggressive behaviors. Parents were also invited to provide qualitative descriptive comments. Note that the questionnaire probed for changes in behavior following engagement in the probed activities; thus, results do not simply reflect the fact that children were unable to engage in symptomatic behaviors such as hand-flapping during the activity. The sets of behavioral changes probed were drawn from relatively broad domains, as the goal in the present study was to sample from a wide set of possible symptom domains relevant for ASDs. While the data are thus an initial test of this relationship, they may be helpful in elucidating symptoms and activities of interest for subsequent research.

Responses were checked to ensure consistency between quantitative data and qualitative comments. For each activity, ratings were averaged across the nine domains to give a summary score; a score of 3 would indicate no change in behavior subsequent to the activity, and scores of 1 and 5 would indicate significant decline or improvement, respectively. These average behavioral change scores were transformed into a grand mean for adenosine-increasing or non-increasing activities (Table 1). Not all participants provided information in order to generate both scores (e.g., they may not have had more than one adenosine-stimulating activity). All available data are included in analyses as indicated.

A grand mean was available for both the increasing and non-increasing categories for 155 participants. A repeated-measures ANOVA on adenosine-increasing and non-increasing activities indicated a significant effect of activity, $F_{1,158} = 108.7$, $p < 0.001$, $\eta_p^2 = 0.41$. Adenosine-increasing activities received a mean score of