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Adenosine and Autism: A Spectrum of Opportunities

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Abstract

In rodents, insufficient adenosine produces behavioral and physiological symptoms consistent with several comorbidities of autism. In rodents and humans, stimuli postulated to increase adenosine can ameliorate these comorbidities. Because adenosine is a broad homeostatic regulator of cell function and nervous system activity, increasing adenosine's influence might be a new therapeutic target for autism with multiple beneficial effects.

Introduction

Autism Spectrum Disorder (ASD) and its associated symptomatology have been associated with abnormalities in several neurotransmitters. The strongest findings involve serotonin, blood concentrations of which are elevated in many autistic patients (Buitelaar and Willemsen-Swinkels, 2000). Other studies or hypotheses have implicated catecholamines (Beverdors, 2010; Rylander, 1972; Yoo et al., 2009), amino acid transmitters (Fatemi et al., 2009; Purcell et al., 2001), and peptide transmitters (Bartz and Hollander, 2008). While many mechanisms may be involved, here we suggest that the neuromodulator adenosine could play a role in ameliorating neurological and behavioral sequelae of ASD.

A diagnosis of ASD relies on three core behavioral symptoms, irrespective of cognitive function: deficits in sociability; deficits in communication; and stereotyped and/or repetitive behavior (American Psychiatric Association, 2000). There is also a constellation of common comorbidities, including epilepsy, disturbed sleep, anxiety, immune dysfunctions, and gastrointestinal problems (Landrigan, 2010). The increasing prevalence of ASD is in itself

alarming, and it is accompanied by a parallel increase in these chronic comorbidities (Hertz-Picciotto et al., 2006; Siegel et al., 2010).

Despite postulated neurochemical abnormalities, and intensive research efforts, at this time there is no biologically-validated treatment related directly to core symptoms of autism (Lam et al., 2006). Treatments are prescribed primarily to limit symptoms (such as seizures or anxiety) with the hope that reducing co-morbidities could facilitate general improvements or be beneficial for core symptoms of the disorder. This frustrating lack of direct medical treatments for ASD is likely due to a host of factors such as diverse underlying genetic and environmental interactions, individual differences in the expression of similar underlying genetics and physiology, and a similar clinical presentation of completely different underlying genetics and physiology. In addition to these genetic and epigenetic complexities, we may be identifying non-essential targets, or producing mixed benefits - symptomatic relief and negative side effects in parallel.

The plethora of underlying causes – and the range of severity or “spectrum” which exemplifies ASD - makes it unlikely that a highly specific and targeted therapy will treat a majority of cases. Rather, ASD may benefit from less selective drugs or interventions that could offer multiple benefits and fewer serious side effects. This type of approach is consistent with comprehensive behavioral intervention, currently the most effective treatment for ASD (LeBlanc and Gillis, 2012). Regarding a broad-based approach with multiple potential benefits, adenosine is a homeostatic bioenergetic regulator of neuronal activity with diverse short- and long-term

effects throughout the body (Boison et al., 2011). Adenosine links to metabolism and to neuronal activity (via ATP/adenine nucleotide pools and actions at G protein-coupled adenosine receptors, respectively). It is present throughout the extracellular space, and both neurons and glia release ATP (which is subsequently dephosphorylated in the extracellular space) or release adenosine directly (Lovatt et al., 2012). Adenosine is removed from the extracellular space primarily by equilibrative transport and phosphorylated intracellularly by adenosine kinase (Dunwiddie and Masino, 2001).

Multiple adenosine receptor agonists are in clinical trials (Gao and Jacobson, 2011), and several currently approved pharmaceuticals have adenosine-modulating properties, such as allopurinol (a xanthine oxidase inhibitor) and dipyridamole (an adenosine transport blocker) (Paterson and Oliver, 1971; Rundles, 1966). While these agents are not typically prescribed with the intent to influence adenosine, recent research and clinical trials have explored this mechanism of action regarding epilepsy (DeMarco and Zagnoni, 1986; Togha et al., 2007; Zagnoni et al., 1994), pain (Connor, 2009), schizophrenia (Akhondzadeh et al., 2000; Brunstein et al., 2001; Dickerson et al., 2009; Lara et al., 2001; Weiser et al., 2012), and bipolar disorder (Akhondzadeh et al., 2006; Fan et al., 2012; Machado-Vieira et al., 2008). As discussed in more detail below, a ketogenic diet has also been shown to activate adenosine receptors (Masino et al., 2011b).

Pharmaceutical treatment for children with autism consists primarily of antipsychotics (Posey et al., 2008); it would seem reasonable to consider therapies with adenosine-based beneficial “side effects.”

Caffeine is a non-selective adenosine A₁/A₂ receptor antagonist, and the most widely used psychoactive drug worldwide (Fredholm et al., 1999). As such, a vast epidemiological database exists regarding manipulation of adenosinergic activity. Regarding the immediate potential for adenosine-based therapies, and in recognition of its safety, accessibility, and neurological effects, researchers have suggested that caffeine itself may be an adjuvant treatment for diverse neurological disorders (Ribeiro and Sebastião, 2010), and there is some historical precedent for this (Fredholm, 2011). As discussed later, caffeine can antagonize adenosine receptors with acute administration or upregulate adenosine receptor signaling with chronic administration (Johansson et al., 1997; Li et al., 2008b). Unfortunately, controlled data on interactions between acute or chronic caffeine and ASD is lacking.

Regarding adenosine modulation and autism, several reports have suggested therapeutic potential with diverse postulated benefits (Freitag et al., 2010; Ghanizadeh, 2010; Masino et al., 2009, 2011a; Tanimura et al., 2010). However, at this time the direction of the relationship, the relevance for particular symptoms, and the receptor subtypes and brain regions involved remain speculative. Here we explore the relationship between adenosine in the central nervous system and autism in terms of clinical symptoms, sensory abnormalities and behavior. We also suggest therapeutic options based on this relationship.

Adenosine and autism – clinical links

Adenosine is known for its role as a sleep-promoter, a seizure-reducer, and a neuroprotective molecule (Dunwiddie and Masino, 2001). All of these roles reflect that adenosine is integral in

the relationship between metabolism and neuronal activity. For example, increased adenosine in specific brain areas is thought to promote sleep as a daily homeostatic metabolic response (Bjorness et al., 2009; Porkka-Heiskanen and Kalinchuk, 2011; Satoh et al., 1998; Stenberg et al., 2003). Similarly, during excess neuronal activity or metabolism, increased adenosine provides a local feedback inhibition which reduces metabolic demand, limits excitability, and protects neurons from energy failure or excitotoxicity (Fredholm, 2007). We hypothesized that augmenting adenosine could alleviate core symptoms and some comorbidities of ASD (Masino et al., 2011a). For instance, adenosine, through A_2 receptors (and possibly by coactivation of A_1 and A_2 receptors), reduces perseverative behaviors in rodents (Poleszak and Manuta, 2000; Tanimura et al., 2010) and could aid with this core symptom in ASD. In a parental survey-based study, conditions and events known to or hypothesized to increase adenosine (e.g. fever, high-intensity physical activity, very low carbohydrate diet) significantly improved autistic behaviors, particularly in Asperger's and verbally-fluent individuals (Masino et al., 2011a).

Because caffeine is known to modulate the influence of adenosine, and because it is so widely used, it is important to consider the interactions and implications of caffeine use in individuals with ASD. Hypotheses regarding adenosine's influence on symptoms of autism suggest that chronic rather than acute caffeine might be most helpful for treating some symptoms and comorbidities. While acute caffeine - at doses ingested typically by humans - transiently blocks a portion of brain adenosine A_1 and A_2 receptors, multiple research reports show that chronic caffeine upregulates the influence of adenosine A_1 receptors somewhat selectively (Green and Stiles, 1986; Li et al., 2008b; Rudolphi et al., 1989) - although not all findings concur on this

point (Johansson et al., 1997). Beyond direct neural effects, there may be an important influence of increased adenosine A₁ receptor activation on microglia and immune function (Chen et al., 2010; Tsutsui et al., 2004). Thus, save for the time period when the acute dose of caffeine is active, the net effect may be increased adenosine A₁ receptor activation. This outcome should be considered with respect to epilepsy therapy given the incontrovertible role of the adenosine A₁ receptor as an anticonvulsant mechanism. In contrast, chronic caffeine may have less of an influence on adenosine A_{2A} receptor regulation (Li et al., 2008b; Lupica et al., 1991), suggesting a continuation of acute effects even with chronic use. The influence of caffeine on adenosine receptors, and the timing, frequency and dose of caffeine use, should be considered with respect to any adenosine-modulating therapy.

Regarding comorbidities, anxiety is a prominent comorbidity in ASD (White et al., 2009), and increased adenosine is anxiolytic (Jain et al., 1995). As for seizures, the evidence that adenosine can decrease or stop them is overwhelming (for instance, Etherington and Frenguelli, 2004; Ilie et al., 2012), and the high prevalence of seizures in children with autism is well-established (Seidenberg et al., 2009; Tuchman and Rapin, 2002). The reported rate of co-morbid seizures in ASD (estimates range from 5-40%) is inevitably conservative: in the absence of behavioral evidence, electroencephalographic (EEG) recordings are not performed routinely in ASD. In addition to the possibility of undetected EEG abnormalities there remains the likelihood that subcortical or localized abnormal EEG activity or microseizures may be occurring, also without any behavioral indication, and such events may be undetectable with surface electrodes. For example, these types of non-behavioral seizures observed only with implanted electrodes have

been observed in several mouse models of adenosine deficiency and Alzheimer's disease (Li et al., 2008a; Masino et al., 2011b; Palop et al., 2007). Regardless of the cause of frank seizures or covert seizure activity, increasing adenosine would be predicted to be an effective strategy in mitigating this abnormal activity.

More recently, diverse neurological disorders such as Parkinson's disease, schizophrenia and chronic pain have been linked to or noted as treated by adenosine-based mechanisms (Burnstock et al., 2011). Increased adenosine is analgesic (Herrick-Davis et al., 1989; Jain et al., 1995) whereas decreased adenosine causes hyperalgesia (Johansson et al., 2001). Regarding schizophrenia and ASD, schizophrenia has recently been postulated to be an adult-onset type of autism. This comparison is due to a number of commonalities including gray matter reductions in a partially overlapping set of regions (Cheung et al., 2010), and similar deficits in brain connectivity, mirror neuron function, and theory of mind (King and Lord, 2011). In parallel, over the last decade an adenosine hypothesis of schizophrenia has been evolving, in which abnormal adenosine levels allow overactivity of dopaminergic systems (leading to positive symptoms, e.g. hallucinations) and underactivity of glutamatergic systems (leading to negative symptoms, e.g. social withdrawal) (Boison et al., 2012; Lara and Souza, 2000). Recently, adenosine-releasing grafts were shown to reverse behavioral and cognitive impairments in an anatomically specific manner in a mouse model of schizophrenia (Shen et al., 2012). These results linking adenosine and schizophrenia may have implications for ASD and more work is needed.

Adenosine and autism – sensory abnormalities

Sensory abnormalities are common in ASD, and persons with autism report these as the most distressing aspects of their disorder (Grandin, 2009). There have been multiple studies which show that children on the autism spectrum not only suffer from sensory abnormalities, but are more likely to suffer from more than one of these abnormalities compared to other children with developmental and language disabilities (Kientz and Dunn, 1996; Leekam et al., 2007). These abnormalities can manifest in children with ASD being either over- or under-reactive to sensory input. In a study of 37 children with autism, it was found that 13 displayed sensory defensiveness as part of their pathology (Kern et al., 2001). Sensory defensiveness is defined as a low threshold for sensory input, and results in avoidant behaviors and adverse reactions to seemingly innocuous environmental occurrences or contact (Kern et al., 2006). As a result, clothes and their tags can be uncomfortable and certain textures or tastes of foods are avoided and can lead to a severely restricted diet. In addition, bright lights are disturbing, and various noises, many noises and loud noises are abnormally distressing to someone suffering from a low sensory threshold (Kern et al., 2001).

In a study conducted by Rosenhall et al. (1999), autistic children were compared to controls in auditory processing, and 18% of the autistic participants were found to be especially sensitive to sounds, a stark contrast to the controls where none were found to have abnormal perception (n=199). Another study found that every autistic child under age three had auditory sensory abnormalities compared to a control group (n = 26; Dahlgren and Gillberg, 1989). Finally, in a comprehensive study conducted on 104 autistic children and matched controls, it was found that the autistic participants had abnormal auditory, oral, tactile and visual sensory

processing; the younger the subject, the more pronounced the abnormalities based on scores on the Sensory Profile (Kern et al., 2006). Behaviors that may develop to adapt to or cope with hyper- and hypo-sensitive processing common in ASD are potentially maladaptive.

With respect to adenosine and sensory processing, research has shown that adenosine disruption or augmentation is associated with specific sensory effects. For example, the shake reflex in rats, elicited by immersion in ice water or injection of icilin, was suppressed by an injection of adenosine or 2-chloroadenosine, a metabolically stable adenosine analogue (Tse and Wei, 1986). This result suggests that adenosine plays a role in inhibiting somatosensory processing, which then causes changes in behavior. In mice, a spinal injection of adenosine was shown to have antinociceptive effects, and receptor antagonists induced a pain response, supporting the hypothesis that adenosine plays a role in inhibiting sensory perception (Keil and DeLander, 1996). Consistent with this, allopurinol was shown to reduce pain via increasing the actions of adenosine at the A₁ receptor subtype (Schmidt et al., 2009).

Adenosine influences sensory processing in other systems as well. In the auditory system adenosine receptors are found in cochlear tissues, and adenosine A₁ receptor expression in the cochlea has been shown to increase under auditory stress, perhaps serving a protective role (Ramkumar et al., 2004; Vlajkovic et al., 2007). In the visual system, adenosine suppresses exocytosis in retinal cone terminals by decreasing Ca²⁺ channel activity, thus reducing light transduction, and is neuroprotective for both cones and rods (Stella et al., 2010).

Together, these studies indicate that augmentation of adenosine can reduce sensory sensitivity, and a deficiency in adenosine levels or receptors could result in altered thresholds of sensitivity in sensory processing for at least touch, vision and hearing. Combined with the profuse evidence of the presence of sensory abnormalities experienced in individuals on the autism spectrum, adenosine augmentation emerges as a strong candidate for the investigation into the treatment of these symptoms.

Adenosine and autism - metabolic therapy

Metabolic therapies include pharmacological and/or physiological strategies, and dietary treatments and/or supplements. Dietary treatments for ASD are many, but few offer either controlled clinical studies or clear evidence of efficacy. Despite this, dietary approaches remain popular with parents, who currently have no safe and effective pharmacological option to address core symptoms. Even though there is little evidence that these diets are specifically helping with symptom of autism, evidence is mounting consistently that a whole food diet is associated with less depression and anxiety and a general sense of well-being. In contrast, a poor diet was associated with negative mental health outcomes such as depression and anxiety (Jacka et al., 2010, 2011; Sánchez-Villegas et al., 2011). These recent findings include large prospective studies, both genders, and adolescents and adults. Therefore, a healthy diet would seem to be helpful mentally and physically, and could complement other therapeutic interventions.

Regarding evidence-based medicine, historically fasting was a metabolic treatment for a wide variety of illnesses and ailments (Kerndt et al., 1982), and epilepsy provides an example of a clear relationship between fasting and brain function. Fasting can stop seizures, but the necessary termination of the fast often precipitated a seizure shortly thereafter (Wheless, 2008). These observations of the relationship between fasting and seizures were validated subsequently by a metabolic therapy using a dietary approach. A ketogenic diet is a high fat, low carbohydrate, adequate protein formula which, similar to fasting, promotes the use of ketones (rather than glucose) for energy. This dietary protocol has been validated as effective as stopping seizures in retrospective, prospective, multi-center, and randomized studies (Hassan et al., 1999; Neal et al., 2008; Vining et al., 1998). The ketogenic diet has been in use for over 90 years, and even the earliest reports noted dramatic efficacy in refractory seizures in children and adults, and also noted anti-seizure effects that outlasted the administration of the diet itself – suggesting long-term disease modification (Wilder, 1921a, b).

In current clinical practice there is continuing evidence for lasting changes – children can be on the diet for 6 months to 2 years, gradually weaned off the diet and often have no ongoing dietary restrictions and remain seizure-free (Martinez et al., 2007). In a prospective study, children who were randomly assigned the ketogenic diet experienced fewer seizures (and some became seizure-free) whereas children who continued with the current standard of care with antiepileptic drugs gradually become worse - experienced increased seizures over time, and none became seizure-free (Neal et al., 2008). If long term improvement or recovery (similar to

epilepsy) could be mobilized for autism - either with a metabolic dietary approach or an analogous pharmacotherapy, it would address a major treatment gap.

Recently it was shown that a KD increases the actions of adenosine at adenosine A₁ receptors to stop electrographic seizures (Masino et al., 2011b), and an in vitro mimic of the ketogenic diet similarly revealed adenosine-based neuronal inhibition (Kawamura et al., 2010). A key aspect of this in vitro mimic is sufficient intracellular ATP and reduced extracellular glucose, two metabolic features of the ketogenic diet. A separate study in hippocampal slices demonstrated an analogous finding: supplementing artificial CSF with d-ribose and adenine increased adenine nucleotide levels and increased extracellular adenosine sufficient to alter activity-dependent long-term potentiation in hippocampal slices and influenced synaptic plasticity (zur Nedden et al., 2011). A survey of parents of children with autism showed that stimuli postulated to increase adenosine can ameliorate symptoms of autism (Masino et al., 2011a). These studies suggest that diverse metabolic approaches can reliably increase adenosine signaling.

Although the advent of antiepileptic pharmaceuticals diminished general interest in dietary therapy, ketogenic diets remain an important option for pediatric and refractory epilepsy. Their particular success with pediatric epilepsy, and long term outcomes, make the diet an option that should seriously be considered in conditions where seizures are co-morbid, such as autism. Due to multiple confounding factors, treatment-resistant epilepsy in autism is associated with very poor outcomes (Sansa et al., 2011).

Currently, there are few studies regarding the effects of the KD on autistic behavior. There has been only one prospective study of the effects of an intermittent ketogenic diet on the behavior of children with autism (Evangelidou et al., 2003). This pilot study was conducted on 30 children four to ten years old. The diet was applied intermittently for six months and parents were asked to evaluate their child's behavior using the Childhood Autism Rating Scale. Of the group that maintained the diet (18 of 30 children = 60%), two children reported a significant improvement (>12 units on the scale), eight patients an average improvement (8-12 units) and eight children a mild improvement (2-8 units). The biggest improvements were noticed in those patients who showed only mild autistic behavior. Nevertheless, these results are promising.

In a pilot study of seven girls with Rett syndrome (which primarily affects girls and presents with dementia, repetitive hand movements, a lack of speech, and severe seizures), parental reports revealed that the diet caused small improvements in behavior, sociability, and mood in five out of seven subjects (Haas et al., 1986), suggesting behavioral improvements that would be beneficial for ASD. In agreement with this clinical finding, a study by Mantis et al. (2009) described ketogenic diet-induced improvements in emotional withdrawal, hesitance, and anxiety in the MeCP2^{308/y} mouse model of Rett syndrome. However, as the study included a standard diet-restricted group with similar behavioral improvements, the authors concluded that the neuroprotective effects stemmed primarily from a reduction in total calorie intake rather than caloric origin. Other effects of the KD do not rely on caloric restriction, however (Ruskin et al., 2009). More research is needed in this area.

Of clinical importance considering the high comorbidity of autism and epilepsy, significant autistic behaviors in a naturalistic mouse model of epilepsy (the EL mouse) have been quantified (Meidenbauer et al., 2011). Previously, seizures in this model was shown to be reduced by a calorically-restricted standard or ketogenic diet (Mantis et al., 2004), and their onset delayed by a ketogenic diet (Todorova et al., 2000). Recently we found that a ketogenic diet reduced autistic behaviors in in the BTBR T+ tf/J mouse, a non-epileptic model of autism (Masino et al., 2012). These and other models may provide new platforms to test hypotheses and therapies that target core symptoms of autism.

Overall, metabolic approaches to treating neurological disorders are gaining credibility for several reasons: (1) metabolic dysfunction is found with neurological dysfunction, although is it typically unclear which came first; (2) the neurological dysfunction associated with epilepsy has been proven to be treated with metabolic therapy; (3) data are emerging regarding the ability of a ketogenic diet to treat other acute and chronic disorders beyond epilepsy (spinal cord injury, neurodegenerative disorders). Autism could benefit significantly from hypothesis-based metabolic approaches.

Conclusions

Basic and clinical research suggests a meaningful relationship between adenosine and treating ASD. The nature of that relationship deserves more research. Meanwhile, the prevalence of autism diagnoses has been increasing at an alarming rate - far outpacing increases in any other developmental disorder (Centers for Disease Control and Prevention, 2012) - and the source of

this continued increase is not well understood. Regardless of the cause, new treatments are needed.

Taken together, emerging evidence suggests that pursuing a hypothesis-based, evidence-based rationale regarding the potential for adenosine-modulating therapies for autism is ready for clinical implementation. For patients with refractory epilepsy, a ketogenic diet might offer multiple benefits. For adult patients with autism, currently approved drugs could be tested immediately, and other options may soon be on the horizon.

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