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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 (Beversdorf, 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 A_1/A_2 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 A_1 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 A_1 receptor activation. This outcome should be considered with respect to epilepsy therapy given the incontrovertible role of the adenosine A_1 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 A_1 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 A_1 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 Ca^{2+} 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 A_1 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 (Evangeliou 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.

References

Akhondzadeh, S., Milajerdi, M.R., Amini, H., Tehrani-Doost, M., 2006. Allopurinol as an adjunct to lithium and haloperidol for treatment of patients with acute mania: a double-blind, randomized, placebo-controlled trial. Bipolar Disord. 8, 485-489.

Akhondzadeh, S., Shasavand, E., Jamilian, H., Shabestari, O., Kamalipour, A., 2000. Dipyridamole in the treatment of schizophrenia: adenosine-dopamine receptor interactions. J. Clin. Pharm. Ther. 25, 131-137.

American Psychiatric Association, 2000. Diagnostic and Statistical Manual of Mental Disorders IV, Text Revision. American Psychiatric Association, Washington DC.

Bartz, J.A., Hollander, E., 2008. Oxytocin and experimental therapeutics in autism spectrum disorders. Prog. Brain Res. 170, 451-462.

Beversdorf, D.Q., 2010. The role of the noradrenergic system in autism spectrum disorders. In: Blatt, G.J., (Ed), Neurochemical Basis of Autism: From Molecules to Minicolumns. Springer, New York, pp. 175-184.

Bjorness, T.E., Kelly, C.L., Gao, T., Poffenberger, V., Greene, R W., 2009. Control and function of the homeostatic sleep response by adenosine A_1 receptors. J. Neurosci. 29, 1267-1276.

Boison, D., Masino, S.A., Geiger, J.D., 2011. Homeostatic bioenergetic network regulation: a novel concept to avoid pharmacoresistance in epilepsy. Expert Opin. Drug Discov. 6, 713-724.

Boison, D., Singer, P., Shen, H.-Y., Feldon, J., Yee, B.K., 2012. Adenosine hypothesis of schizophrenia - Opportunities for pharmacotherapy. Neuropharmacology 62, 1527-1543.

Brunstein, M.G., Belmonte-de-Abreu, P., Souza, D.O., Lara, D.R., 2001. Therapeutic benefit of adjunctive dipyridamole in schizophrenia is probably due to adenosine-glutamate interactions. J. Clin. Pharm. Ther. 26, 155-156.

Buitelaar, J.K., Willemsen-Swinkels, S. H., 2000. Medication treatment in subjects with autistic spectrum disorders. Eur. Child Adolesc. Psychiatry 9 Suppl. 1, 85-97.

Burnstock, G., Krügel, U., Abbracchio, M.P., Illes, P., 2011. Purinergic signalling: from normal behaviour to pathological brain function. Prog. Neurobiol. 95, 229-274.

Centers for Disease Control and Prevention, 2012. Prevalence of autism spectrum disorders--autism and developmental disabilities monitoring network, 14 sites, United States, 2008. Morbidity and Mortality Weekly Report 61, 1-19.

Chen, G.Q., Chen, Y.Y., Wang, X.S., Wu, S.Z., Yang, H.M., Xu, H.Q., He, J.C., Wang, X.T., Chen, J.F., Zheng, R.Y., 2010. Chronic caffeine treatment attenuates experimental autoimmune encephalomyelitis induced by guinea pig spinal cord homogenates in Wistar rats. Brain Res. 1309, 116-125.

Cheung, C., Yu, K., Fung, G., Leung, M., Wong, C., Li, Q., Sham, P., Chua, S., McAlonan, G., 2010. Autistic disorders and schizophrenia: related or remote? An anatomical likelihood estimation. PLoS One 5, e12233.

Connor, M., 2009. Allopurinol for pain relief: more than just crystal clearance? Br. J. Pharmacol. 156, 4-6.

Dahlgren, S.P., Gillberg, C., 1989. Symptoms in the first two years of life: A preliminary population study of infantile autism. Eur. Arch. Psychiatry Neurol. Sci. 238, 169-174.

DeMarco, P., Zagnoni, P., 1986. Allopurinol and severe epilepsy. Neurology 36, 1538-1539.

Dickerson, F.B., Stallings, C.R., Origoni, A.E., Sullens, A., Khushalani, S., Sandson, N., Yolken, R.H., 2009. A double-blind trial of adjunctive allopurinol for schizophrenia. Schizophr. Res. 109, 66-69.

Dunwiddie, T.V., Masino, S.A., 2001. The role and regulation of adenosine in the central nervous system. Annu. Rev. Neurosci. 24, 31-55.

Etherington, L.A., Frenguelli, B.G., 2004. Endogenous adenosine modulates epileptiform activity in rat hippocampus in a receptor subtype-dependent manner. Eur. J. Neurosci. 19, 2539-2550.

Evangeliou, A., Vlachonikolis, I., Mihailidou, H., Spilioti, M., Skarpalezou, A., Makaronas, N., Prokopiou, A., Christodoulou, P., Liapi-Adamidou, G., Helidonis, E., Sbyrakis, S., Smeitink, J., 2003. Application of a ketogenic diet in children with autistic behavior: pilot study. J. Child Neurol. 18, 113-118.

Fan, A., Berg, A., Bresee, C., Glassman, L.H., Rapaport, M.H., 2012. Allopurinol augmentation in the outpatient treatment of bipolar mania: a pilot study. Bipolar Disord. 14, 206-210.

Fatemi, S.H., Reutiman, T.J., Folsom, T.D., Thuras, P.D., 2009. $GABA_A$ receptor downregulation in brains of subjects with autism. J. Autism Dev. Disord. 39, 223-230.

Fredholm, B.B., 2007. Adenosine, an endogenous distress signal, modulates tissue damage and repair. Cell Death Differ. 14, 1315-1323.

Fredholm, B.B., 2011. Notes on the history of caffeine use. Handb. Exp. Pharmacol. (200), 1-9.

Fredholm, B.B., Battig, K., Holmen, J., Nehlig, A., Zvartau, E.E., 1999. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacol. Rev. 51, 83-133.

Freitag, C.M., Agelopoulos, K., Huy, E., Rothermundt, M., Krakowitzky, P., Meyer, J., Deckert, J., von Gontard, A., Hohoff, C., 2010. Adenosine A_{2A} receptor gene (ADORA2A) variants may increase autistic symptoms and anxiety in autism spectrum disorder. Eur. Child Adolesc. Psychiatry 19, 67-74.

Gao, Z.G., Jacobson, K.A., 2011. Emerging adenosine receptor agonists: an update. Expert Opin. Emerg. Drugs 16, 597-602.

Ghanizadeh, A., 2010. Possible role of caffeine in autism spectrum disorders, a new testable hypothesis. J. Food Sci. 75, ix.

Grandin, T., 2009. Visual abilities and sensory differences in a person with autism. Biol. Psychiatry 65, 15-16.

Green, R.M., Stiles, G.L., 1986. Chronic caffeine ingestion sensitizes the A_1 adenosine receptor-adenylate cyclase system in rat cerebral cortex. J. Clin. Invest. 77, 222-227.

Haas, R.H., Rice, M.A., Tauner, D.A., Merritt, T.A., 1986. Therapeutic effects of a ketogenic diet in Rett syndrome. Am. J. Med. Genet. Suppl. 1, 225-246.

Hassan, A.M., Keene, D.L., Whiting, S.E., Jacob, P.J., Champagne, J.R., Humphreys, P., 1999. Ketogenic diet in the treatment of refractory epilepsy in childhood. Pediatr. Neurol. 21, 548-552.

Herrick-Davis, K., Chippari, S., Luttinger, D., Ward, S.J., 1989. Evaluation of adenosine agonists as potential analgesics. Eur. J. Pharmacol. 162, 365-369.

Hertz-Picciotto, I., Croen, L., Hansen, R., Jones, C., van der Water, J., Pessah, I.N., 2006. The CHARGE study: an epidemiologic investigation of genetic and environmental factors contributing to autism. Environ. Health Perspect. 114, 1119-1125.

Ilie, A., Raimondo, J.V., Akerman, C.J., 2012. Adenosine release during seizures attenuates $GABA_A$ receptor-mediated depolarization. J. Neurosci. 32, 5321-5332.

Jacka, F.N., Kremer, P.J., Berk, M., de Silva-Sanigorski, A.M., Moodie, M., Leslie, E.R., Pasco, J.A., Swinburn, B.A., 2011. A prospective study of diet quality and mental health in adolescents. PLoS One 6, e24805.

Jacka, F.N., Pasco, J.A., Mykeltun, A., Williams, L.J., Hodge, A.M., O'Reilly, S.L., Nicholson, G.C., Kotowicz, M.A., Berk, M., 2010. Association of Western and traditional diets with depression and anxiety in women. Am. J. Psychiatry 167, 305-311.

Jain, N., Kemp, N., Adeyemo, O., Buchanan, P., Stone, T.W., 1995. Anxiolytic activity of adenosine receptor activation in mice. Br. J. Pharmacol. 116, 2127-2133.

Johansson, B., Georgiev, V., Lindström, K., Fredholm, B.B., 1997. A_1 and A_{2A} adenosine receptors and A_1 mRNA in mouse brain: effect of long-term caffeine treatment. Brain Res. 762, 153-164.

Johansson, B., Halldner, L., Dunwiddie, T.V., Masino, S.A., Poelchen, W., Giménez-Llort, L., Escorihuela, L.M., Fernández-Teruel, A., Wiesenfeld-Hallin, Z., Xu, X.-J., Hårdemark, A., Betscholtz, C., Herlenius, E., Fredholm, B.B., 2001. Hyperalgesia, anxiety, and decreased hypoxic neuroprotection in mice lacking the adenosine A_1 receptor. Proc. Natl. Acad. Sci. USA 98, 9407-9412.

Kawamura, M., Jr., Ruskin, D.N., Masino, S.A., 2010. Metabolic autocrine regulation of neurons involves cooperation among pannexin hemichannels, adenosine receptors and K_{ATP} channels. J. Neurosci. 30, 3886-3895.

Keil, G.J., DeLander, G.E., 1996. Altered sensory behaviors in mice following manipulation of endogenous spinal adenosine neurotransmission. Eur. J. Pharmacol. 312, 7-14.

Kern, J.K., Miller, V.S., Cauller, L., Kendall, R., Mehta, J., Dodd, M., 2001. Effectiveness of N,N-dimethylglycine in autism and pervasive developmental disorder. J. Child Neurol. 16, 169-173.

Kern, J.K., Trivedi, M.H., Barver, C.R., Grannemann, B.D., Andrews, A.A., Savla, J.S., Johnson, D.G., Mehta, J.A., Schroeder, J.L., 2006. The pattern of sensory processing abnormalities in autism. Autism 10, 480-494.

Kerndt, P.R., Naughton, J.L., Driscoll, C.E., Loxterkamp, D.A., 1982. Fasting: the history, pathophysiology and complications. West. J. Med. 137, 379-399.

Kientz, M.A., Dunn, W., 1996. A comparison of the performance of children with and without autism on the sensory profile. Am. J. Occup. Ther. 51, 530-537.

King, B.H., Lord, C., 2011. Is schizophrenia on the autism spectrum? Brain Res. 1380, 34-41.

Lam, K.S., Aman, M.G., Arnold, L.E., 2006. Neurochemical correlates of autistic disorder: a review of the literature. Res. Dev. Disabil. 27, 254-289.

Landrigan, P.J., 2010. What causes autism? Exploring the environmental contribution. Curr. Opin. Pediatr. 22, 219-225.

Lara, D.R., Brunstein, M.G., Ghisolfi, E.S., Lobato, M.I., Belmonte-de-Abreu, P., Souza, D.O., 2001. Allopurinol augmentation for poorly responsive schizophrenia. Int. Clin. Psychopharmacol. 16, 235-237.

Lara, D.R., Souza, D.O., 2000. Schizophrenia: a purinergic hypothesis. Med. Hypotheses 54, 157-166.

LeBlanc, L.A., Gillis, J.M., 2012. Behavioral interventions for children with autism spectrum disorders. Pediatr. Clin. North Am. 59, 147-164.

Leekam, S.R., Nieto, C., Libby, S.J., Wing, J., Gould, J., 2007. Describing the sensory abnormalities of children and adults with autism. J. Autism Dev. Disord. 37, 894-910.

Li, T., Ren, G., Lusardi, T., Wilz, A., Lan, J.Q., Iwasato, T., Itohara, S., Simon, R.P., Boison, D., 2008a. Adenosine kinase is a target for the prediction and prevention of epileptogenesis in mice. J. Clin. Invest. 118, 571-582.

Li, W., Dai, S., An, J., Li, P., Chen, X., Xiong, R., Liu, P., Wang, H., Zhao, Y., Zhu, M., Liu, X., Zhu, P., Chen, J.-F., Zhou, Y., 2008b. Chronic but not acute treatment with caffeine attenuates traumatic brain injury in the mouse cortical impact model. Neuroscience 151, 1198-1207.

Lovatt, D., Xu, Q., Liu, W., Takano, T., Smith, N.A., Schnermann, J., Tieu, K., Nedergaard, M., 2012. Neuronal adenosine release, and not astrocytic ATP release, mediates feedback inhibition of excitatory activity. Proc. Natl. Acad. Sci. USA 109, 6265-6270.

Lupica, C.R., Berman, R.F., Jarvis, M.F., 1991. Chronic theophylline treatment increases adenosine A1 but not A2 receptor binding in the rat brain: an autoradiographic study. Synapse 9, 95-102.

Machado-Vieira, R., Soares, J.C., Lara, D.R., Luckenbaugh, D.A., Busnello, J.V., Marca, G., Cunha, A., Souza, D.O., Zarate, C.A., Jr., Kapczinski, F., 2008. A double-blind, randomized, placebo-

controlled 4-week study on the efficacy and safety of the purinergic agents allopurinol and dipyridamole adjunctive to lithium in acute bipolar mania. J. Clin. Psychiatry 69, 1237-1245.

Mantis, J.G., Centeno, N.A., Todorova, M.T., McGowan, R., Seyfried, T.N., 2004. Management of multifactorial idiopathic epilepsy in EL mice with caloric restriction and the ketogenic diet: role of glucose and ketone bodies. Nutr. Metab. 1, 11.

Mantis, J.G., Fritz, C.L., Marsh, J., Heinrichs, S.C., Seyfried, T.N., 2009. Improvement in motor and exploratory behavior in Rett syndrome mice with restricted ketogenic and standard diets. Epilepsy Behav. 15, 133-141.

Martinez, C.C., Pyzik, P.L., Kossoff, E.H., 2007. Discontinuing the ketogenic diet in seizure-free children: recurrence and risk factors. Epilepsia 48, 187-190.

Masino, S.A., Kawamura, M., Jr., Plotkin, L.M., Svedova, J., DiMario, F.J., Jr., Eigsti, I.-E., 2011a. The relationship between the neuromodulator adenosine and behavioral symptoms of autism. Neurosci. Lett. 500, 1-5.

Masino, S.A., Kawamura, M., Jr., Wasser, C.D., Pomeroy, L.T., Ruskin, D.N., 2009. Adenosine, ketogenic diet and epilepsy: the emerging therapeutic relationship between metabolism and brain activity. Curr. Neuropharmacol. 7, 257-268.

Masino, S.A., Li, T., Theofilas, P., Ruskin, D.N., Fredholm, B.B., Geiger, J.D., Aronica, E., Boison, D., 2011b. A ketogenic diet suppresses seizures in mice through adenosine A_1 receptors. J. Clin. Invest. 121, 2679-2683.

Masino, S.A., Svedova, J., Cote, J.L., Kawamura, M., Jr., Ruskin, D.N., 2012. Ketogenic diet reverses autistic behaviors in BTBR mouse model of autism. Society for Neuroscience, San Diego, California.

Meidenbauer, J.J., Mantis, J.G., Seyfried, T.N., 2011. The EL mouse: a natural model of autism and epilepsy. Epilepsia 52, 347-357.

Neal, E.G., Chaffe, H., Schwartz, R.H., Lawson, M.S., Edwards, N., Fitzsimmons, G., Whitney, A., Cross, J.H., 2008. The ketogenic diet for the treatment of childhood epilepsy: a randomised controlled trial. Lancet Neurol. 7, 500-506.

Palop, J.J., Chin, J., Roberson, E.D., Wang, J., Thwin, M.T., Bien-Ly, N., Yoo, J., Ho, K.O., Yu, G.-Q., Kreitzer, A., Finkbeiner, S., Noebels, J.L., Mucke, L., 2007. Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. Neuron 55, 697-711.

Paterson, A.R.P., Oliver, J.M., 1971. Nucleoside transport: II. Inhibition by *p*-nitrobenzylthioguanosine and related compounds. Can. J. Biochem. 49, 271-274.

Poleszak, E., Manuta, D., 2000. Influence of adenosine receptor agonists and antagonists on amphetamine-induced stereotypy in rats. Pol. J. Pharmacol. 52, 423-429.

Porkka-Heiskanen, T., Kalinchuk, A.V., 2011. Adenosine, energy metabolism and sleep homeostasis. Sleep Med. Rev. 15, 123-135.

Posey, D.J., Stigler, K.A., Erickson, C.A., McDougle, C.J., 2008. Antipsychotics in the treatment of autism. J. Clin. Invest. 118, 6-14.

Purcell, A.E., Jeon, O.H., Zimmerman, A.W., Blue, M.E., Pevsner, J., 2001. Postmortem brain abnormalities of the glutamate neurotransmitter system in autism. Neurology 57, 1618-1628.

Ramkumar, V., Whitworth, C.A., Pingle, S.C., Hughes, L.F., Rybak, L.P., 2004. Noise induces A_1 adenosine receptor expression in the chinchilla cochlea. Hear. Res. 188, 47-56.

Ribeiro, J.A., Sebastião, A.M., 2010. Caffeine and adenosine. J. Alzheimers Dis. 20 (Suppl. 1), S3-S15.

Rosenhall, U., Nordin, V., Sandström, M., Ahlsén, G., Gillberg, C., 1999. Autism and hearing loss. J. Autism Dev. Disord. 29, 349-357.

Rudolphi, K.A., Keil, M., Fastbom, J., Fredholm, B.B., 1989. Ischaemic damage in gerbil hippocampus is reduced following upregulation of adenosine (A_1) receptors by caffeine treatment. Neurosci. Lett. 103, 275-280.

Rundles, R.W., 1966. Metabolic effects of allopurinol and allo-xanthine. Ann. Rheum. Dis. 25 (6 Suppl.), 615-620.

Ruskin, D.N., Kawamura, M., Jr., Masino, S.A., 2009. Reduced pain and inflammation in juvenile and adult rats fed a ketogenic diet. PLoS One 4, e8349.

Rylander, G., 1972. Psychosis and the punding and choreiform syndromes in addiction to central stimulant drugs. Folia Psychiatr. Neurol. Neurochir. 75, 203-212.

Sánchez-Villegas, A., Verberne, L., De Irala, J., Ruíz-Canela, M., Toledo, E., Serra-Majem, L., Martínez-González, M.A., 2011. Dietary fat intake and the risk of depression: the SUN Project. PLoS One 6, e16268.

Sansa, G., Carlson, C., Doyle, W., Weiner, H.L., Bluvstein, J., Barr, W., Devinsky, O., 2011. Medically refractory epilepsy in autism. Epilepsia 52, 1071-1075.

Satoh, S., Matsumura, H., Hayaishi, O., 1998. Involvement of adenosine A2A receptor in sleep promotion. Eur. J. Pharmacol. 351, 155-162.

Schmidt, A.P., Böhmer, A.E., Antunes, C., Schallenberger, C., Porciúncula, L.O., Elisabetsky, E., Lara, D.R., Souza, D.O., 2009. Anti-nociceptive properties of the xanthine oxidase inhibitor allopurinol in mice: role of A_1 adenosine receptors. Br. J. Pharmacol. 156, 163-172.

Seidenberg, M., Pulsipher, D.T., Hermann, B., 2009. Association of epilepsy and comorbid conditions. Future Neurol. 4, 663-668.

Shen, H.-Y., Singer, P., Lytle, N., Wei, C., Lan, J.-Q., Williams-Karnesky, R.L., Chen, J.-F., Yee, B.K., Boison, D., 2012. Adenosine augmentation ameliorates psychotic and cognitive endophenotypes of schizophrenia in mice. J. Clin. Invest. 122, 2567-2577.

Siegel, B., Ihle, E., Marco, E., Hendren, R., 2010. Update on autism: issues in treatment and comorbidity. Psychiatr. Times 27, 33-39.

Stella, S. L., Jr., Hu, W.D., Brecha, N.C., 2010. Adenosine suppresses exocytosis from cone terminals of the salamander retina. NeuroReport 20, 923-929.

Stenberg, D., Litonius, E., Halldner, L., Johansson, B., Fredholm, B.B., Porkka-Heiskanen, T., 2003. Sleep and its homeostatic regulation in mice lacking the adenosine A_1 receptor. J. Sleep Res. 12, 283-290.

Tanimura, Y., Vaziri, S., Lewis, M.H., 2010. Indirect basal ganglia pathway mediation of repetitive behavior: Attenuation by adenosine receptor agonists. Behav. Brain Res. 210, 116-122.

Todorova, M.T., Tandon, P., Madore, R.A., Stafstrom, C.E., Seyfried, T.N., 2000. The ketogenic diet inhibits epileptogenesis in EL mice: a genetic model for idiopathic epilepsy. Epilepsia 41, 933-940.

Togha, M., Akhondzadeh, S., Motamedi, M., Ahmadi, B., Razeghi, S., 2007. Allopurinol as adjunctive therapy in intractable epilepsy: a double-blind and placebo-controlled trial. Arch. Med. Res. 38, 313-316.

Tse, S.Y., Wei, E.T., 1986. Inhibition of the shake response in rats by adenosine and 2-chloroadenosine. Psychopharmacology 90, 322-326.

Tsutsui, S., Schnermann, J., Noorbakhsh, F., Henry, S., Yong, V.W., Winston, B.W., Warren, K., Power, C., 2004. A1 adenosine receptor upregulation and activation attenuates neuroinflammation and demyelination in a model of multiple sclerosis. J. Neurosci. 24, 1521-1529.

Tuchman, R., Rapin, I., 2002. Epilepsy in autism. Lancet Neurol. 1, 352-358.

Vining, E.P., Freeman, J.M., Ballaban-Gil, K., Camfield, C.S., Camfield, P.R., Holmes, G.L., Shinnar, S., Shuman, R., Trevathan, E., Wheless, J.W., 1998. A multicenter study of the efficacy of the ketogenic diet. Arch. Neurol. 55, 1433-1437.

Vlajkovic, S.M., Abi, S., Wang, C.J., Housley, G.D., 2007. Differential distribution of adenosine receptors in rat cochlea. Cell Tissue Res. 328, 461-471.

Weiser, M., Gershon, A.A., Rubinstein, K., Petcu, C., Ladea, M., Sima, D., Podea, D., Keefe, R.S., Davis, J.M., 2012. A randomized controlled trial of allopurinol vs. placebo added on to antipsychotics in patients with schizophrenia or schizoaffective disorder. Schizophr. Res. 138, 35-38.

Wheless, J.W., 2008. History of the ketogenic diet. Epilepsia 49 suppl. 8, 3-5.

White, S.W., Oswald, D., Ollendick, T., Scahill, L., 2009. Anxiety in children and adolescents with autism spectrum disorders. Clin. Psychol. Rev. 29, 216-229.

Wilder, R.M., 1921a. The effects of ketonemia on the course of epilepsy. Mayo Clin. Bull. 2, 307.

Wilder, R.M., 1921b. High fat diets in epilepsy. Mayo Clin. Bull. 2, 308.

Yoo, H.J., Lee, S.K., Park, M., Cho, I.H., Hyun, S.H., Lee, J.C., Yang, S.Y., Kim, S.A., 2009. Family- and population-based association studies of monoamine oxidase A and autism spectrum disorders in Korean. Neurosci. Res. 63, 172-176.

Zagnoni, P.G., Bianchi, A., Zolo, P., Canger, R., Cornaggia, C., D'Alessandro, P., DeMarco, P., Pisani, F., Gianelli, M., Verze, L., et al., 1994. Allopurinol as add-on therapy in refractory epilepsy: a double-blind placebo-controlled randomized study. Epilepsia 35, 107-112.

zur Nedden, S., Hawley, S., Pentland, N., Hardie, D.G., Doney, A.S., Frenguelli, B.G., 2011. Intracellular ATP influences synaptic plasticity in area CA1 of rat hippocampus via metabolism to adenosine and activity-dependent activation of adenosine A_1 receptors. J. Neurosci. 31, 6221-6234.