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Bumetanide in Children and Adolescents with Autism Spectrum Disorder

Running Title: Bumetanide and Autism Spectrum Disorder

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Highlights

- An imbalance in excitatory/inhibitory functions in the early development of brain can be considered as an etiological factor in developing autism spectrum disorder.
- In the early development of brain, GABA acts as an excitatory agent which leads to the accumulation of chloride inside the cell. Bumetanide, a loop diuretic, is an antagonist to NKCC1 receptor, which can reduce the intracellular chloride and shift GABA from excitation to inhibition state.
- In several studies, it has been shown that bumetanide can reduce the symptoms of the autism spectrum disorder and can be considered to be a new treatment for this disorder.

Plain Language Summary

Autism spectrum disorder (ASD) is regarded as a neurodevelopmental disorder characterized by impairments in fulfilling social relationships with others along with having repetitive behaviors and interests. The prevalence rate of ASD has increased in recent years. As well, the main interventions used for this disorder have been mainly non-pharmacological. There are also a few medications such as Risperidone and Aripiprazole approved for reducing some non-core symptoms of ASD. Besides, there is no cure improvement for social interactions in these individuals. Recently, Bumetanide has been introduced as a new medication to ameliorate the core symptoms of ASD e.g. relatedness to others. Bumetanide has been correspondingly utilized in medicine as a diuretic for several years. Some studies have also reported that the given medication can improve deficits in children with ASD. Considering its mechanism, Bumetanide seems to influence the main symptoms of ASD such as social relationships. Thus, the present article reviewed the studies reporting efficacy and adverse effects of Bumetanide in individuals with ASD.

Abstract

Introduction: Autism spectrum disorder (ASD) is characterized by several impairments in communications and social interactions as well as restricted interests or stereotyped behaviors. Interventions applied for this disorder are based on multi-modal approaches, including pharmacotherapy. No cure or medication has been introduced so far. Therefore, there were studies investigating several drugs for treating individuals with ASD. One of the recent medications introduced for this purpose was Bumetanide. Accordingly, the purpose of the present article was to review the efficacy of this drug on the core symptoms of ASD and its potential side effects.

Method: All papers reported pharmacokinetics, pharmacodynamics, efficacy, and adverse effects of Bumetanide on animal models and humans with ASD. The papers were reviewed based on the main databases of PubMed, Web of Science, and Scopus.
Results: The findings revealed that cortical neurons have high chloride ion $(Cl^-)_i$ and excitatory actions of Gamma Aminobutyric Acid (GABA) in the valporic acid animal model with ASD and mice with fragile X syndrome. Bumetanide which has firstly been introduced as a diuretic is also a high affinity-specific Na^+-K^+-Cl^- cotransporter (NKCC1) antagonist that can reduce (Cl^-). The results also indicated that Bumetanide could attenuate behavioral features of autism in both animal and human models. Moreover, the studies showed that such a medication could activate fusiform face area in individuals with ASD while viewing emotional faces. Also, recent findings suggested that the dose of 1 mg of this drug taken twice daily might be the best compromise between safety and efficacy.
Conclusion: Recent studies provided pieces of evidence that Bumetanide could be considered to be a novel pharmacological agent in treating core symptoms of ASD. Future studies need to be conducted in order to confirm the efficacy of this medication in individuals with ASD.
Keywords: Bumetanide, Diuretics, Autism spectrum disorder

1. Introduction

Autism is a neurodevelopmental disorder in children characterized by impairments in communications and social interactions as well as limited and repetitive patterns of behaviors and interests (Dilip Jeste et al., 2012). Recent investigations have also estimated the prevalence rates of 1 in 88 children in the United States and 1 in 54 boys which have elevated within 2 decades ago ("Prevalence of Autism Spectrum Disorders — Autism and Developmental Disabilities Monitoring Network, 14 Sites, United States, 2008," 2012). To date, the United States Federal and Drug Administration (FDA) or European Medicines Agency (EMA) has approved no drugs and effective medical treatments, significantly improving the core symptoms of ASD. However, two authorized medicines to treat autism-related irritability are Risperidone (5-16 years old) and Aripiprazole (6-17 years old). Neither Risperidone; a dopamine type 2 (D2) and serotonin type 2A (5-HT2A) receptor

antagonist (McCracken, 2002), nor Aripiprazole; a partial dopamine (DA) D2 and serotonin 1A receptor (5-HT1A) agonist, and a 5-HT2A antagonist (Wink, Erickson, & McDougle, 2010), have failed to manage the core symptoms of ASD (E. Lemonnier et al., 2017) and both have been found to indicate some side effects, including sedation, vomiting, extrapyramidal syndromes, increased appetite, drowsiness, drooling, and body weight gain (Fung et al., 2016;(E. Lemonnier et al., 2017). Bumetanide, a diuretic and chloride cotransporter antagonist, has been proposed recently as a new therapeutic strategy (E. Lemonnier et al., 2012; Eric Lemonnier & Ben-Ari, 2010). There is much evidence demonstrating that the level of chloride and GABAergic signaling, change in animal models of ASD, individuals suffering from ASD, and other developmental disorders (Yehezkel Ben-Ari, 2015). Bumetanide has been widely used in adults since 1975 and in children from 1986 for the treatment of acute and chronic disorders such as hypertension, nephritic syndrome, blockade-associated heart failure and dysplasia-associated bronchopulmonary which can restore low Cl^-_i levels and shift GABA from excitation to inhibition, therefore it can be also used for a wide range of disorders (Yehezkel Ben-Ari, 2015; Blaesse, Airaksinen, Rivera, & Kaila, 2009; Nardou et al., 2011). This article aimed to bring various studies on Bumetanide and autism together and report the efficacy of this drug in behavioral and cognitive symptoms of ASD.

2. Method

We searched the databases of Web of Science, PubMed, and Scopus. The keywords of the search were ASD, Autism, Asperger, Pervasive Developmental Disorder, and Bumetanide. Moreover, we searched the pharmacological functions of Bumetanide through the mentioned databases. Since the papers in this filed are limited, we did not have any strategy for excluding the papers.

3. Pharmacological Characteristics of Bumetanide

3.1. Chemistry

Bumetanide, a newly emerged diuretic agent, has the same properties of other therapeutic agents related to the class of diuretics affecting the loop such as furosemide and ethacrynic acid (Dixon et al., 1976). It is a 4-substituted derivative of sulfamyl-benzoic acid with the chemical name of 3-nbutylamino-4-phenoxy-5-sulfamyl-benzoic acid. Replacing the chlorine atom with a phenoxy group also identifies Bumetanide from other sulfamoyl diuretics (Figure 1) (Turmen, Thom, Phillip, Louridas, Lemorand, & Arnanda, 1982).

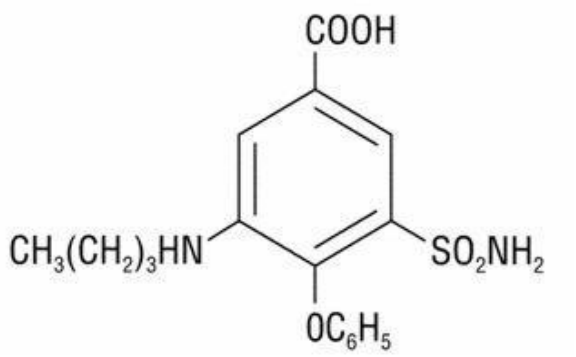

Figure 1. The structural formula of Bumetanide

3.2. Pharmacokinetics

Bumetanide has a short half-life (between 90 minutes and 3 hours) and it can poorly pass through the blood-brain barrier (Li et al., 2011). Following oral administration of 2.0 mg of Bumetanide to healthy volunteers, these general characteristics are observed: approximately full absorption; a peak plasma level at 30 minutes of approximately 80 ng/ml; an obvious half-life of 1.2-1.5 hours; and an average plasma elimination of approximately 228-255 ml/min (Dixon et al., 1976; Pentikäinen, Neuvonen, Kekki, & Penttilä, 1980). The clearance of Bumetanide is triple, with half-life ranging from 6 minutes to 3 hours. It can be also rapidly and almost completely eliminated by urinary excretion and metabolism (Dixon et al., 1976). The metabolism of Bumetanide occurs through the butyl side chain and alcohol is its main metabolite (Flamenbaum & Friedman, 1982; "Halladay et al., 1975," n.d.).

3.3. Pharmacodynamics

3.3.1. GABA and ASD

The exact molecular mechanisms by which ASDs act are not fully elucidated, however, a number of new studies have indicated several clinical pictures of autism are due to activity-related dysregulation of neural development. Mutations that occur in some voltage-gated and ligand-gated ion channels, which are important in excitability regulation of neurons and calcium ion ($C2^{+}$) signaling pathway have been further linked to ASD (Krey & Dolmetsch, 2007). Anomalies in neuronal excitability during development can also occur due to changes in neurotransmitter systems (Barnby et al., 2005). Among such systems, pieces of evidence supports the deficiency of GABAergic inhibition in autism (Krey & Dolmetsch, 2007; Menold et al., 2001). Patients with autism are often recognized by rearrangements in chromosome 15q11-13, constructing a cluster of genes related to GABA genes, including GABRA5, ABRG3, and GABRB3 (Dykens, Sutcliffe, &

Levitt, 2004; Menold et al., 2001). Polymorphisms in GABRA4 are also associated with autism (Collins et al., 2006; Ma et al., 2005). Likewise, other genes that are important in the differentiation and migration of GABAergic interneurons and regulate the development of GABAergic neurons such as ARX are related to ASD. ARX mutations can also result in epilepsy, movement disorders, cortical malformations, mental retardation, and autism (Friocourt, Poirier, Rakić, Parnavelas, & Chelly, 2006; Turner, Partington, Kerr, Mangelsdorf, & Gecz, 2002). Nevertheless, GABA is considered as a major excitatory neurotransmitter during early development when it results in depolarization of neurons and Ca^{2+} influx through voltage-gated Ca^{2+} channels. It sounds that such GABA-recruited Ca2+ transmissions are critical in a large number of neurodevelopmental aspects, including proliferation, migration, dendritic arborization, and development of Purkinje cell (Represa & Ben-Ari, 2005) .

Provocation of GABA receptors is also crucial in the production of simultaneous concurrent network function and modulation of Ca2+ waves in cortex development (Garaschuk, Linn, Eilers, & Konnerth, 2000; Voigt, Opitz, & De Lima, n.d.). Therefore, a deficit in the function of GABA can lead to disturbance in developing brain which may cause some neurodevelopmental disorders such as ASD.

3.3.2. Bumetanide and GABA

As previously mentioned, GABA as a neurotransmitter inhibiting neural functions in an adult brain, acts as a stimulant in the early stages of the postpartum period. This mechanism is based on the accumulation of chloride inside the cell and a “reversed” chloride gradient in a wide variety of neurons and animal species, especially invertebrates (“Jang et al., 2002,” n.d.; Ye, 2004)

Moreover, extensive studies have shown that the two chloride co-transporters of NKCC1 and KCC2 play an important role in this growth trend. NKCC1 and KCC2 are respectively considered as the most important chloride importers and exporters in a way that intracellular chloride levels are mainly controlled by them (Y. Ben-Ari, Gaiarsa, Tyzio, & Khazipov, 2007; Y Ben-Ari, 2014; E. Lemonnier et al., 2012) The high-affinity-specific NKCC1 antagonist, can reduce Cl^-_i and shift GABA from excitation to inhibition (Blaesse et al., 2009; Nardou et al., 2011). It is notable that oxytocin, which improves social communications (Guastella, Mitchell, & Dadds, 2008), acts by decreasing Cl^-_i levels such as Bumetanide (Tyzio et al., 2006).

3.3.3. Bumetanide and ASD

According to the reviewed studies (table 1), maternal infusion of Bumetanide in the ASD animal model and in mice with fragile X syndrome reduced the physiological levels of Cl^-_i in offspring and consequently regulated electrical and behavioral parameters (Eftekhari et al., 2014; Tyzio et al., 2014). The animal studies also showed that GABAergic signals had changed in mice suffering from fragile X syndrome (Chao et al., 2010; Coghlan et al., 2012; Pizzarelli & Cherubini, 2011; Tabuchi et al., 2007), Recent measurements of intracellular chloride in neurons of mice with fragile X syndrome from birth to adulthood also demonstrated increased levels associated with GABA-stimulating activity (Tyzio et al., 2014). Based on these observations, the effects of Bumetanide on five children with autism were evaluated using 5 standard Infantile Autistic Syndrome (IAS) severity tests, including Childhood Autism Rating Scale (CARS), Aberrant Behavior Checklist (ABC), Clinical Global Impressions (CGI), Repetitive and Restrictive Behavior (RRB), and Regulation Disorder Evaluation Grid (RDEG) (Eric Lemonnier & Ben-Ari, 2010). In this study, the diuretic was used (1 mg / 24 hours - 0.5 mg twice a day) and treatment continued for 3 months and the results suggested that Bumetanide had improved behavioral aspects of IAS with no side effects. Lemonnier et al. in a randomized double-blinded study on 60 children aged 3-11 years with autism or Asperger syndrome receiving placebo or Bumetanide (1 mg/day) for 3 months found that Bumetanide had significantly reduced CARS, CGI, and Autism Diagnostic Observation Schedule (ADOS) values. The side effects were also limited to a mild hypokalemia treated with supplemental potassium. The results confirmed the earlier small-scale open-label pilot study in 2010 and showed that Bumetanide had ameliorated ASD symptoms (E. Lemonnier et al., 2012). Also, an open-trial pilot study on 7 adolescents and young adults with autism assessing the effect of 10 months of treatment with Bumetanide showed an improvement of facial emotion recognition and activation of brain regions involved in, including the inferior occipital cortex and the fusiform gyrus. An increased activity in areas which are responsible for rewards, motivations, and emotions, including nucleus accumbens, amygdala, and cortex orbitofrontal was suggested as well. These results augment the benefits of Bumetanide as a promising treatment to ameliorate social interactions in autism (Hadjikhani et al., 2013). The results of a single case report in 2013 using CARS, ADOS, ABC, RDEG, and RRB before and after treatment showed that daily use of Bumetanide for 3 months in a 10-year-old boy with fragile X syndrome had decreased the intensity of ASD symptoms. As reported in this study, the only side effect was a mild hypokalemia (Eric Lemonnier et al., 2013).

In 2015, Bruning described a 10-year-old girl suffering from ASD, dysplasia of the

Table 1. Behavioral and cognitive effects of Bumetanid studies in autism spectrum disorders

cortex, and a duplication in 15q11.2 locus meanwhile she was epileptic. She indicated an extreme excitement in her behaviors following the previous treatment with Clavazam (a benzodiazepine). In this respect, the treatment led to a specific improvement in sensory behaviors, rigidity, and memory performance (Bruining et al., 2015) In a pilot study using Bumetanide in combination with education (0.5 mg twice a day) on 60 children with autism indicated that the total score of ABC, CARS, and SI (severity of disease) were reduced in both groups after 3 months compared to scores at the pre-treatment stage. The overall scores of ABC and CGI in the combined treatment group were significantly lower than the single treatment one. Although the overall scores and CARS-related item scores in the group of combined treatment were less than treatment group after a 3-month intervention, there were no significant results. Generally, treatment with Bumetanide and ABA training compared to only ABA training, led to more improvements in children with autism. No undesirable effects of Bumetanide were observed (Du et al., 2015).

Study	**Participants**	**Method:** (design, diagnostic measures, intervention, duration**)**	**Outcome**
Tabuchi et al., 2007	Neuroligin-3 R451C KI and neuroligin-3 KO mice	- An animal study - Quantitative Western Blots, Whole-cell recordings in layer 2/3 of the somatosensory (barrel) cortex in acute slices, Dark/light box, Novel home cage activity, Open field arena, Elevated plus maze, and Morris water maze.	- A significant elevation in the expression of two markers for inhibitory synapses (the vesicular GABA- transporter VGAT and the postsynaptic protein gephyrin) in the R451C KI mice, while no difference was observed in VGAT expressions in the KO mice. - These data vigorously demonstrated that a difference in the inhibitory/excitatory balance may participate in pathogenesis of ASDs.
Chao et al., 2010	Mice with MeCP2 deficiency in GABAergic neurons (male Viaat-Mecp22/y mice and male littermate controls)	- An animal study - RT–qPCR, Immunolabelling, mIPSCs, A partition test and modified three-chamber assay, Morris water maze, EEG	- MeCP2 deficiency found in GABAergic neurons led to diminished presynaptic function of GABA release and revealed a multitude of neuropsychiatric phenotypes. - Findings indicated that GABAergic dysfunction is a crucial cause of Rett syndrome and

					autistic phenotypes.
Pizzarelli and Cherubini, 2011	ASD and Fragile X animal models	-	Review; focuses on the implications of an altered GABAergic signaling in neurodevelopmental disorders such as ASDs	-	Lower frequency of GABAergic interneuron networks in the cortex, olfactory bulb, and hippocampus, as well as in the GABA-mediated tonic inhibition. - A dysfunction of the GABAergic signaling early in development and a severe E/I unbalance in neuronal circuits which can account for some of the behavioral deficits observed in ASD patients.
Coghlan et al., 2012	Animal models of ASDs and related disorders.	-	Review of evidence from genetics, molecular neurobiology and systems neuroscience relating to the role of GABA in ASD and related disorders including Fragile X syndrome, Rett syndrome, and Fetal Anticonvulsant Syndrome.	-	The results, showed that some of the subunits of the GABAA receptor system have functional roles in neurodevelopment and there is a GABA deficit in autism, Fragile X syndrome, and Rett syndrome.
Tyzio et al., 2014	Two animal models of autism: rats exposed to valproate in the utero (VPA	-	An experimental study - Bumetanide (10 µM) or oxytocin	-	Acute applications of Bumetanide significantly decreased (Cl–)i in

	rats) and mice having the fragile X mutation (FRX mice)	(1 µM) - Whole-cell voltage clamp recordings, Isolation induced ultrasonic vocalizations, Intracranial EEG recordings	neurons observed in VPA rats and FRX mice. - KCC2 was down-regulated in the hippocampi of VPA rats and FRX mice. - Maternal pretreatment with Bumetanide restored electrophysiologic al and behavioral phenotypes and blocking oxytocin signaling, produced autistic-like electrophysiology and behavior in offsprings
Eftekhari et al., 2014	VPA rats and FRX mice	- An experimental study - Acute applications of Bumetanide (10 µM) or oxytocin (1 µM) (Tyzio et al., 2014). - The social approach-avoidance paradigm, Three-chamber social test	- Adult male VPA rats that treated with Bumetanide prior to birth (maternal pretreatment) displayed improved sociability than age-matched non-treated VPA rats. - Adult male FRX mice indicated a significantly higher number of grooming events (bouts) than wild-type (WT) ittermates. - Bumetanide treatment around delivery attenuated autistic behavioral features in adult

					offspring.
Lemonnier and Ben-Ari, 2010	Five neonates with autism	-	A pilot study ICD-10 criteria 5 standard IAS severity tests, ADI-R, CARS, ABC, CGI, RDEG, and RRB. Bumetanide (1 mg / 24 hours - 0.5 mg twice a day) 3 months	-	Bumetanide improved behavioral aspects of IAS The study reported a significant improvement in IAS with no side effects.
Lemonnier et al., 2012	Sixty children suffering from autism or Asperger syndrome (3–11 years old) in two groups.	-	A double-blind, randomized, placebo-controlled trial CARS, CGI, ADOS G, Placebo or Bumetanide (1 mg daily) 3 months	-	Bumetanide significantly reduced CARS, CGI, and ADOS Values Side effects were limited to a mild hypokalemia. Bumetanide ameliorated the symptoms of ASD and is a promising novel therapeutic agent to treat autism.
Hadjikhani et al., 2013	Seven high-functioning males with ASD	-	An open trial pilot study DSM- IV-TR ADOS, ADI-R, the Autism-Spectrum Quotient (AQ), Empathy Quotient (EQ), WASI, and fMRI, Bumetanide treatment (1 mg/day) 10 months	-	Improvement of emotion face recognition and activation of brain regions involved in. Areas included rewards, motivations and emotions, also showed Increase in activity.
Lemonnier et al., 2013	A 10-year-old Fragile X boy	-	A single case report CARS, ADOS, ABC, RDEG, and	-	Decrease the intensity of autistic symptoms Only side effect

		RRB - Bumetanide treatment twice a day (0.5 mg morning and 0.5 mg evening) - 3 months	was a mild hypokalemia - The results raised the possibility of treating FRX children with Bumetanide with a good benefit/risk ratio.
Bruining et al., 2015	a 10-year-old girl suffering from ASD, dysplasia of the cortex, and a duplication in 15q11.2 locus	- A single case report - Repetitive Behavior Scale–Revised, BRIEF, The digit span of the WISC–III and The spatial span of the Wechsler Nonverbal Scale of Ability, The Rey Auditory Verbal Learning Test, The Rey Visual Design Learning Test, The Amsterdam Neuropsychological Tasks battery, and EEG recordings. - Bumetanide treatment (0.5 mg twice daily) - 6 months	- A specific improvement in sensory behaviors, rigidity, learning and memory performance. - Bumetanide improved neural functioning which supported by changes on resting state EEG. - The power of α frequency elevated following the treatment that may explain another mechanism contributing to the improvement of the patients in behavioral and cognitive functions.
Du et al., 2015	Sixty children with autism (two categories: single treatment and combined treatment categories	- A pilot study - ICD-10 criteria - ABC, CARS, SI, GI, CGI - Bumetanide treatment (0.5 mg twice a day) and ABA training - 3-month	- The total score of ABC, CARS, and SI were reduced in both groups. - The overall scores of ABC and CGI in the combined treatment category were significantly reduced in comparison to the single treatment

			category. - CARS in the combined treatment category was lower than the treatment category but not significantly. - No undesirable effects were found.
Lemonnier et al., 2017	- Eighty-eight patients with ASD (2–18 years old) - Groups received Bumetanide (0.5, 1.0 or 2.0 mg twice daily) or placebo	- A double-blind, randomized, placebo-controlled, multisite dose-ranging study - CARS, SRS, CGI-I, - 3 months	- The most prevalent adverse effects were as follow: hypokalemia, increased urine elimination, loss of appetite, dehydration, and asthenia. - The number and incidence of adverse effects directly correlated with the dose of Bumetanide. - Bumetanide made better ASD core symptoms and exhibited a favorable beneficial/detrimental ratio, especially at 1.0 mg twice a day.

4. Effective Dose and Side Effects of Bumetanide

In a recent study aimed to determine the best dose of Bumetanide in the pediatric population (E. Lemonnier et al., 2017), the results showed that the medication was safe with some side effects associated with diuresis and dehydration. Lemonnier et al. also used Bumetanide twice a day and the results indicated that the frequency and severity of side effects had increased with dose, while there was no clear relationship between dose and efficacy. According to the results of this study, the dose of 1 mg twice a day seemed to be the best agreement between safety and efficacy. As mentioned above and based on the results of most studies, administration of Bumetanide was safe and it only had some adverse effects, which were mainly limited to hypokalemia (Hadjikhani et al., 2013; E. Lemonnier et al., 2012, 2017; Eric Lemonnier et al., 2013; Eric Lemonnier & Ben-Ari, 2010), This adverse effect can be controlled by regular monitoring of the kalemia and kidney function in patients to evaluate the side effects (Hadjikhani et al., 2013). Hypokalemia was also clinically manageable with potassium supplementation, dose stabilization, and precise hydration of patients (E. Lemonnier et al., 2017). In other words, the changes observed after taking Bumetanide (and other potent diuretics), including hypokalemia, hypochloremia, hypochloremic metabolic alkalosis, and hyperuricemia (without gastro-esophageal arthritis). It should be noted that biochemical tests commonly used for drug safety did not show any important disturbances other than transitory thrombocytopenia or granulocytopenia (Flamenbaum & Friedman, 1982).

5. Conclusion

ASD is known as a neurodevelopmental disorder characterized by an obvious excitatory/inhibitory imbalance in selective neuronal circuits, particularly in the GABAergic signaling. Accordingly, numerous studies have indicated that drugs affecting GABAergic synapses can ameliorate behavioral defects in animal models of autism and improve at least some of the symptoms observed in ASD patients. Experimental works and clinical observations have further shown that Bumetanide as a safe loop diuretic (E. Lemonnier et al., 2017), may be suggested as a new treatment strategy that ameliorates ASD symptoms without serious adverse effects. Thus, future clinical trials are needed to confirm the efficacy of Bumetanide in ASD.

6. Ethical Considerations

Since the paper is a review paper there was no ethical issues apart from considering the rights of the authors.

7. Compliance with ethical guidelines

There aren`t any ethical guidelines.

8. Funding

This paper did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

9. Conflict of interest

The authors declared no conflict of interest.

References:

Barnby, G., Abbott, A., Sykes, N., Morris, A., Weeks, D. E., Mott, R., … Monaco, A. P. (2005). Candidate-Gene Screening and Association Analysis at the Autism-Susceptibility Locus on Chromosome 16p: Evidence of Association at GRIN2A and ABAT. *The American Journal of Human Genetics*. https://doi.org/10.1086/430454

Ben-Ari, Y. (2014). NEUROSCIENCE FOREFRONT REVIEW THE GABA EXCITATORY/INHIBITORY DEVELOPMENTAL SEQUENCE: A PERSONAL JOURNEY. *Neuroscience*, *279*, 187–219. https://doi.org/10.1016/j.neuroscience.2014.08.001

Ben-Ari, Y. (2015). Is birth a critical period in the pathogenesis of autism spectrum disorders? *Nature Reviews Neuroscience*. https://doi.org/10.1038/nrn3956

Ben-Ari, Y., Gaiarsa, J.-L., Tyzio, R., & Khazipov, R. (2007). GABA: A Pioneer Transmitter That Excites Immature Neurons and Generates Primitive Oscillations. *Physiological Reviews*. https://doi.org/10.1152/physrev.00017.2006

Blaesse, P., Airaksinen, M. S., Rivera, C., & Kaila, K. (2009). Cation-Chloride Cotransporters and Neuronal Function. *Neuron*. https://doi.org/10.1016/j.neuron.2009.03.003

Bruining, H., Passtoors, L., Goriounova, N., Jansen, F., Hakvoort, B., de Jonge, M., & Poil, S.-S. (2015). Paradoxical Benzodiazepine Response: A Rationale for Bumetanide in Neurodevelopmental Disorders? *PEDIATRICS*. https://doi.org/10.1542/peds.2014-4133

Chao, H. T., Chen, H., Samaco, R. C., Xue, M., Chahrour, M., Yoo, J., … Zoghbi, H. Y. (2010). Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes. *Nature*. https://doi.org/10.1038/nature09582

Coghlan, S., Horder, J., Inkster, B., Mendez, M. A., Murphy, D. G., & Nutt, D. J. (2012). GABA system dysfunction in autism and related disorders: From synapse to symptoms. *Neuroscience and Biobehavioral Reviews*. https://doi.org/10.1016/j.neubiorev.2012.07.005

Collins, A. L., Ma, D., Whitehead, P. L., Martin, E. R., Wright, H. H., Abramson, R. K., … Pericak-Vance, M. A. (2006). Investigation of autism and GABA receptor subunit genes in multiple ethnic groups. *Neurogenetics*. https://doi.org/10.1007/s10048-006-0045-1

Dilip Jeste, P. V, Jeffrey Lieberman, P.-E. A., David Fassler, T., Rcxser Peele, S., Speaker Scott Benson, A. R., Akaka, J., … Erik R Vanderlip, P. D. (2012). American Psychiatric Association Board of Trustees Member-in-Training Trustee-Elect.

Dixon, W. R., Young, R. L., Holazo, A., Jack, M. L., Weinfeld, R. E., Alexander, K., … Kaplan, S. A. (1976). Bumetanide: Radioimmunoassay and pharmacokinetic profile in humans. *Journal of Pharmaceutical Sciences*. https://doi.org/10.1002/jps.2600650518

Du, L., Shan, L., Wang, B., Li, H., Xu, Z., Staal, W. G., & Jia, F. (2015). A Pilot Study on the Combination of Applied Behavior Analysis and Bumetanide Treatment for Children with Autism. *Journal of Child and Adolescent Psychopharmacology*. https://doi.org/10.1089/cap.2015.0045

Dykens, E. M., Sutcliffe, J. S., & Levitt, P. (2004). Autism and 15Q11-Q13 disorders: Behavioral, genetic, and pathophysiological issues. *Mental Retardation and Developmental Disabilities Research Reviews*. https://doi.org/10.1002/mrdd.20042

Eftekhari, S., Shahrokhi, A., Tsintsadze, V., Nardou, R., Brouchoud, C., Conesa, M., … Science, sciencemagorg. (2014). Response to Comment on " Oxytocin-mediated GABA inhibition during delivery attenuates autism pathogenesis in rodent offspring ". *Science Neuropharmacology Neuropsychopharmacology Brain Res. Science PLOS ONE Genes Brain Behav. J. Peça et Al. Nature Transl. Psychiatry*, *343*(346), 750–760.

Flamenbaum, W., & Friedman, R. (1982). Pharmacology, Therapeutic Efficacy, and Adverse Effects of Bumetanide, A New “Loop” Diuretic. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*. https://doi.org/10.1002/j.1875-9114.1982.tb03188.x

Friocourt, G., Poirier, K., Rakić, S., Parnavelas, J. G., & Chelly, J. (2006). The role of ARX in cortical development. *European Journal of Neuroscience*. https://doi.org/10.1111/j.1460-9568.2006.04629.x

Fung, L. K., Mahajan, R., Nozzolillo, A., Bernal, P., Krasner, A., Jo, B., … Hardan, A. Y. (2016). Pharmacologic Treatment of Severe Irritability and Problem Behaviors in Autism: A Systematic Review and Meta-analysis. *PEDIATRICS*, *137*(2). Retrieved from http://pediatrics.aappublications.org/content/pediatrics/137/Supplement_2/S124.full.pdf

Garaschuk, O., Linn, J., Eilers, J., & Konnerth, A. (2000). Large-scale oscillatory calcium waves in the immature cortex. *Nature Neuroscience*. https://doi.org/10.1038/74823

Guastella, A. J., Mitchell, P. B., & Dadds, M. R. (2008). Oxytocin Increases Gaze to the Eye Region of Human Faces. *Biological Psychiatry*.

https://doi.org/10.1016/j.biopsych.2007.06.026

Hadjikhani, N., Zürcher, N. R., Rogier, O., Ruest, T., Hippolyte, L., Ben-Ari, Y., & Lemonnier, E. (2013). Improving emotional face perception in autism with diuretic bumetanide: A proof-of-concept behavioral and functional brain imaging pilot study. *Autism*. https://doi.org/10.1177/1362361313514141

Halladay et al., 1975. (n.d.).

Jang et al., 2002. (n.d.).

Krey, J. F., & Dolmetsch, R. E. (2007). Molecular mechanisms of autism: a possible role for Ca2+ signaling. *Current Opinion in Neurobiology*. https://doi.org/10.1016/j.conb.2007.01.010

Lemonnier, E., & Ben-Ari, Y. (2010). The diuretic bumetanide decreases autistic behaviour in five infants treated during 3 months with no side effects. *Acta Paediatrica, International Journal of Paediatrics*. https://doi.org/10.1111/j.1651-2227.2010.01933.x

Lemonnier, E., Degrez, C., Phelep, M., Tyzio, R., Josse, F., Grandgeorge, M., … Ben-Ari, Y. (2012). A randomised controlled trial of bumetanide in the treatment of autism in children. *Translational Psychiatry*. https://doi.org/10.1038/tp.2012.124

Lemonnier, E., Robin, G., Degrez, C., Tyzio, R., Grandgeorge, M., & Ben-Ari, Y. (2013). Treating Fragile X syndrome with the diuretic bumetanide: A case report. *Acta Paediatrica, International Journal of Paediatrics*. https://doi.org/10.1111/apa.12235

Lemonnier, E., Villeneuve, N., Sonie, S., Serret, S., Rosier, A., Roue, M., … Ben-Ari, Y. (2017). Effects of bumetanide on neurobehavioral function in children and adolescents with autism spectrum disorders. *Translational Psychiatry*. https://doi.org/10.1038/tp.2017.10

Li, Y., Cleary, R., Kellogg, M., Soul, J. S., Berry, G. T., & Jensen, F. E. (2011). Sensitive isotope dilution liquid chromatography/tandem mass spectrometry method for quantitative analysis of bumetanide in serum and brain tissue. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*. https://doi.org/10.1016/j.jchromb.2011.02.018

Ma, D. Q., Whitehead, P. L., Menold, M. M., Martin, E. R., Ashley-Koch, A. E., Mei, H., … Pericak-Vance, M. A. (2005). Identification of Significant Association and Gene-Gene Interaction of GABA Receptor Subunit Genes in Autism. *Am. J. Hum. Genet*, *77*, 377–388.

McCracken, J. (2002). The New Eng land Jour nal of Medicine RISPERIDONE IN CHILDREN WITH AUTISM AND SERIOUS BEHAVIORAL PROBLEMS A BSTRACT Background Atypical antipsychotic agents, which. *N Engl J Med*, *347*(5), 314–21. https://doi.org/10.1056/NEJMoa013171

Menold, M. M., Shao, Y., Wolpert, C. M., Donnelly, S. L., Raiford, K. L., Abramson, R. K., … Gilberta, J. R. (2001). ASSOCIATION ANALYSIS OF SUBUNIT GENES IN AUTISTIC DISORDER CHROMOSOME 15 GABAA RECEPTOR, *15*, 3–4.

Nardou, R., Yamamoto, S., Chazal, G., Bhar, A., Ferrand, N., Dulac, O., … Khalilov, I. (2011). Neuronal chloride accumulation and excitatory GABA underlie aggravation of neonatal epileptiform activities by phenobarbital. *Brain*. https://doi.org/10.1093/brain/awr041

Pentikäinen, P. J., Neuvonen, P. J., Kekki, M., & Penttilä, A. (1980). Pharmacokinetics of intravenously administered bumetanide in man. *Journal of Pharmacokinetics and Biopharmaceutics*. https://doi.org/10.1007/BF01059643

Pizzarelli, R., & Cherubini, E. (2011). Alterations of GABAergic signaling in autism spectrum disorders. *Neural Plasticity*. https://doi.org/10.1155/2011/297153

Prevalence of Autism Spectrum Disorders — Autism and Developmental Disabilities Monitoring Network, 14 Sites, United States, 2008. (2012). *Surveillance Summaries*, *61*(3). Retrieved from https://www.cdc.gov/mmwr/pdf/ss/ss6103.pdf

Represa, A., & Ben-Ari, Y. (2005). Trophic actions of GABA on neuronal development. *Trends in Neurosciences*. https://doi.org/10.1016/j.tins.2005.03.010

Tabuchi, K., Blundell, J., Etherton, M. R., Hammer, R. E., Liu, X., Powell, C. M., & Südhof, T. C. (2007). A neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice. *Science*. https://doi.org/10.1126/science.1146221

Turmen, T., Thom, Phillip, Louridas, A. T., Lemorand, P., & Arnanda, J. V. (1982). Protein Binding and Bilirubin Displacing Properties of Bumetanide and Furosemide. *The Journal of Clinical Pharmacology*. https://doi.org/10.1002/j.1552-4604.1982.tb02648.x

Turner, G., Partington, M., Kerr, B., Mangelsdorf, M., & Gecz, J. (2002). Variable expression of mental retardation, autism, seizures, and dystonic hand movements in two families with an identical ARX gene mutation. *American Journal of Medical Genetics*. https://doi.org/10.1002/ajmg.10714

Tyzio, R., Cossart, R., Khalilov, I., Minlebaev, M., Hübner, C. A., Represa, A., … Khazipov, R. (2006). Maternal oxytocin triggers a transient inhibitory switch in GABA signaling in the fetal brain during delivery. *Science*. https://doi.org/10.1126/science.1133212

Tyzio, R., Nardou, R., Ferrari, D. C., Tsintsadze, T., Shahrokhi, A., Eftekhari, S., … Ben-Ari, Y. (2014). Oxytocin-mediated GABA inhibition during delivery attenuates autism pathogenesis in rodent offspring. *Science*. https://doi.org/10.1126/science.1247190

Voigt, T., Opitz, T., & De Lima, A. D. (n.d.). Synchronous Oscillatory Activity in Immature Cortical Network Is Driven by GABAergic Preplate Neurons.

Wink, L. K., Erickson, C. A., & McDougle, C. J. (2010). Pharmacologic treatment of behavioral symptoms associated with autism and other pervasive developmental disorders. *Current Treatment Options in Neurology*. https://doi.org/10.1007/s11940-010-0091-8

Ye, J.-H. (2004). Presynaptic Glycine Receptors on GABAergic Terminals Facilitate Discharge of Dopaminergic Neurons in Ventral Tegmental Area. *Journal of Neuroscience*. https://doi.org/10.1523/JNEUROSCI.2016-04.2004