Intranasal Vasopressin — A Potential Drug Proven to Control the Symptoms and Behaviours of Autism Spectrum Disorder: A Review

By

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A thesis submitted to the School of Pharmacy in partial fulfilment of the requirements for the degree of Bachelor of Pharmacy (B. Pharm.)

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing a degree at Brac University.

2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited full and accurate referencing.

3. The thesis does not contain material that has been accepted or submitted, for any other degree or diploma at a university or other institution.

4. I have acknowledged all main sources of help.

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Approval

The thesis titled “Intranasal Vasopressin, A Potential Drug Proven To Control The Symptoms And Behaviours Of Autism Spectrum Disorder: A Review” submitted by Adeeba Noor Alam (18146094) of Spring 2022 has been accepted as satisfactory in partial fulfilment of the requirement for the degree of Bachelor of Pharmacy.

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Ethics statement

This study does not involve any kind of animal trial or human trial.

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Abstract

Autism spectrum disorder (ASD) is a neurodevelopmental disorder marked by impaired socio-emotional behaviours, communication, motor functions, hyper-fixation and sensory overstimulation, and has received much attention since the last century with major technological advances and destigmatisation movements, yet its exact causes or treatment remaining unclear. Currently, arginine vasopressin has been suggested as playing some definitive role in causing ASD, and in this review work, the studies conducted to understand its possible influence on appropriate social and cooperative behaviour and relevant literature has been included. These articles focused on the administration of arginine vasopressin (AVP) to animal and human participants and evaluated the effects of AVP in ASD core or associative symptoms using various outcome measure tests, such as Social Communication and Interaction (SCI), Social Responsiveness Scale (SRS), Restricted Interests and Repetitive Behaviour Scale (RRB), Facial Emotion Recognition Test (FERT), etc. and the research findings yielded favourable results for ASD patients. It was confirmed that vasopressin did indeed affect neural networks and AVP-administered groups had performed better than the control groups in ASD treatment drug trials, with minimal adverse effects and good tolerability and safety margin.

Keywords: Autism spectrum disorder, neurodevelopmental, socio-emotional behaviours, arginine vasopressin, core or associative symptoms, outcome measure tests, neural network, tolerability.

Table of Contents

List of Tables

List of Figures

List of Acronyms

ASD	Autism Spectrum Disorder
AVP	Arginine vasopressin
AVPR1A/V1aR	Arginine vasopressin-receptor 1A
AVPR1B/V1bR	Arginine vasopressin-receptor 1B
AVPR2	Arginine vasopressin-receptor 2
CGI-I	Clinical Global Impression-Improvement
CGI-S	Clinical Global Impression-Severity
DSM	Diagnostic and Statistical Manual of Mental Disorders
ECG	Electrocardiogram
FDA	Food and Drug Administration
FERT	Facial Emotion Recognition Test
GPCR	G-protein coupled receptors
NEPSY	Developmental Neurodevelopmental Assessment
NDA	New drug application
n/s	Not significant
OXTR	Oxytocin receptor
RBS-R	Repetitive Behaviour Scale Revised
RMET	Reading the Mind in the Eyes Test

RRB	Restricted Interests and Repetitive Behaviour Scale
SCAS	Spence Children's Anxiety Scale
SCI	Social Communication and Interaction Scale
SRS-2 T	Social Responsiveness Scale-2
SSRI	Selective Serotonin Reuptake Inhibitors
TEAS	Trans-cutaneous Electrical Acupoint Stimulation
WHO	World Health Organisation

Chapter 1: Introduction

1.1 Autism Spectrum Disorder

Autism spectrum disorder is a group of neurodevelopmental disorders that are known to cause deficits in the areas of social communication as well as intelligence. It can manifest as excessively repetitive actions, non-verbal habits, misreading of social cues and hyper fixation on certain interest(s) as defined by Merriam-Webster's dictionary (Merriam-Webster, 2022). It includes Pervasive Developmental Disorder Not Otherwise Specified (PDD-NOS), Asperger's syndrome and Childhood Disintegrative Disorder. ASD onsets during the developmental and early years of a child's life. These symptoms can vary from mild to severe, mainly thought to be occurring in the two domains of "social communication, restrictive repetitive behaviours/interests"- whether it be conceptual, social or practical.

Occurring in 1% of the world's population (World Health Organisation, 2021), there is much stigma associated with being on the spectrum, not only for the patient but also their families, whether it being stereotyped, isolated or discriminated against for a condition that is completely out of their control.

1.2 Causes of Autism Spectrum Disorder

While the exact cause of ASD remains largely unknown, many studies have pointed to the fact that it may be a resultant effect of several factors. While predisposition, disorders and mutations related to genes are regarded as a decisive cause of autism, other factors, such as the foetus being exposed to certain medications, heavy metals or other environmental toxins (e.g.: mercury, thalidomide, valproic acid, etc.), parents being older when conceiving or the mother being overly-exposed to viral infections can also lead to a child developing autism (Ornoy et al., 2015; Chaste & Leboyer, 2012).

Christensen et al. has noted ASD to be 4.3 times more likely to affect boys than girls as of 2016, however, it has been hypothesised that ASD in girls simply presents differently and is more prone to being masked, leading to fewer reports in girls than boys (Christensen et al., 2016).

1.3 Global Impact of ASD

Baxter et al. had presented in an epidemiology study that as of 2010, 52 million cases of autism had been estimated globally, roughly prevailing in around 1-2% of the total world population. Their study had also found that in children under five years of age, ASD was the leading cause of disabilities rooted in mental disorders, and due to the fact that there is currently limited epidemiological and clinical evidence pertaining to ASD remission, it is generally regarded as being a life-long condition (Baxter et al., 2014).

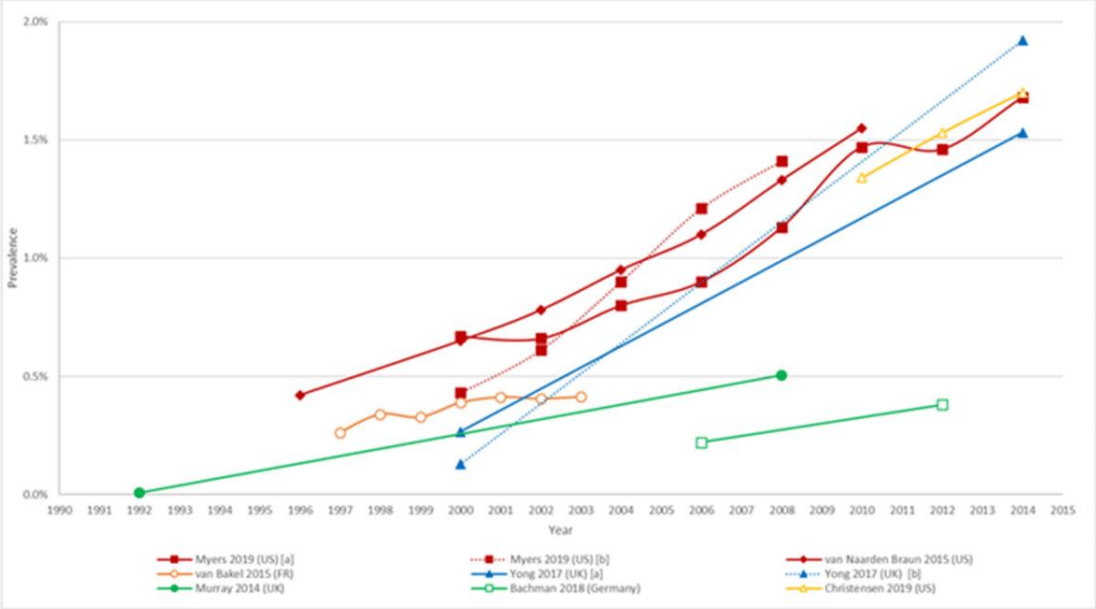

Fig 1: Trends in prevalence of ASD from 1992 to 2014 (Bougeard et al. 2021).

In the 2010 Global Burden of Disease, ASD was included as one of the twenty leading causes of disabilities (World Bank, 1983), outranking attention-deficit hyperactivity disorder (ADHD)

with a total disability-adjusted life year (DALYs) of 7.7 million versus 6.2 million (Erskine et al., 2014).

While geographic, cultural, socio-economic or ethnic factors cannot be particularly pinpointed as causation, they have been associated with delayed diagnosis and inaccessibility to treatment facilities, mainly stemming from mental health issues being stigmatised in many countries and communities (Durkin et al., 2012; Elsabbagh et al., 2012). Lower middle-income countries are staring in the face of more severe challenges and deficit research which is keeping their ASD population deprived of comprehensive care and opportunities when compared to the strides that are being made in higher-income countries, despite the former hosting a larger ASD population than the latter. However, in both settings, there are struggles that they both face when it comes to the diagnosis and treatment of ASD, irrespective of the country's economic status. Proper usage of instruments, financial burden, societal adaptation, lack of resources as well as highly trained healthcare professionals continues to pose significant hurdles in the endeavour to treat ASD (World Health Organisation, 2013).

1.4 Diagnosis of Autism Spectrum Disorder

Specific clinical traits have been historically recorded and are currently being used to this day for the diagnosis of ASD. According to the American Psychiatric Association, these include but are not limited to identifying: a) intellectual or vocabulary deficit; b) accompanying medical or genetic conditions; c) inability to reach various development milestones- cognitive, social, intellectual, etc. skills at the correct age; d) deficits in social-emotional reciprocity, unable to maintain relationships, deficient non-verbal communication (abnormal body language, avoidance of eye contact); e) repetitive patterns or habits- strictly adhering to routines, hyper fixation, hyper-reactivity or hypo-reactivity or under/overstimulation to the environment (sounds, textures, lights); f) symptoms are prone to be masked in later stages of

life as to “fit in with society” g) debilitates day to day life (American Psychiatric Association, 2013).

1.6 Currently available treatments to control ASD

Anxiety and aggressive tendencies are the most prominent symptoms associated with ASD (Neumann & Landgraf, 2012; White et al., 2009). Currently, drugs such as selective serotonin reuptake inhibitors (SSRIs), antipsychotics and anticonvulsants (Potenza & McDougle, 1997) are being used to treat ASD associative-behaviours, by targeting dysfunctional neurotransmitters, unbalanced neuronal pathways, genetic mutations, etc. and thus help manage it to an extent (Berry-Kravis et al., 2018; Weele et al., 2017; Williams et al., 2010).

However, these have not been FDA-approved specifically for the treatment of ASD (U.S. Food and Drug Administration, 2022), as they have merely been fruitful in suppressing associated symptoms, rather than core symptoms. Some of them are discouraged for use in paediatric patients as they can cause negative side effects and they cannot target and cure ASD itself.

1.7 Research gap

In recent times, vasopressin and oxytocin-based options are being explored and developed as studies have shown their promise in the field of ASD treatment. Their pathways have shown a connection with the causation as well as promise in treating various symptoms unique to ASD. However, this is a fairly new course of action and despite research papers implying that vasopressin can indeed be effectively utilised in ASD treatment, clinical trials are still in their infancy and are ongoing.

1.8 Aim and Objectives of the Study

The aim of this study was to review the current literature available on the administration of

intranasal vasopressin on individuals with autism spectrum disorder.

The objectives were to:

I. Explore and scrutinise the information detailing the effects of intranasal vasopressin.

II. Find out the mechanism of action of how it helps to improve ASD symptoms in a patient.

III. Explore its future prospects.

Chapter 2: Methodology

All the terms corresponding to arginine vasopressin, autism spectrum disorder and their related information were searched, explored and compiled from various websites such as Google Scholar, NCBI, SpringerLink, PubMed, Elsevier, etc. and the journals found from them. Around 85 articles were referenced and their abstracts were screened by investigating the terms arginine vasopressin, vasopressin receptor agonist, autism spectrum disorder, impaired social behaviours due to ASD, treatment of ASD, outcome measure tests, etc. and their methods and results were reviewed. Supplementary articles relevant to the topics were included for usage and non-relevant articles were excluded. From the selected articles, their contents and results were reviewed and data extracted and collected by focusing on the specific topic. Finally, the project paper was written by analysing the accumulated information.

Chapter 3: Findings and Discussion

The World Health Organisation (WHO) reports that ASD is a genetic condition that affects about 1 in 160 children worldwide (World Health Organisation, 2021) and causes intellectual and communication disabilities in those affected. While many high-functioning patients can be simply medicated and live their lives as healthy people, there still remains a great number of those with ASD who are wholly dependent on their families and caregivers for support to perform in some cases even basic tasks.

While our society's perception of privilege and treatment of those who are less fortunate is definitely a debate to be had, the scientific community's concern is focused on treating or curing ASD to help rehabilitate and help them function better as human beings.

3.1 Vasopressin

Albers describe arginine vasopressin (1-desamino-8-D-arginine-vasopressin) or antidiuretic hormone as a peptide prohormone primarily associated in the body with water reabsorption in the excretory system as well as functions in the blood circulatory system and influencing social and reproductive behaviours (especially in males) (Albers, 2012). Synthesized in the hypothalamus and released in response to stress, dehydration, uterine dilatation and sexual stimulation, vasopressin acts as a neuromodulator (Born et al., 2002) and helps regulate the processing of social information and behaviours in mammals (Meyer-Lindenberg et al., 2011). If sexual stimulation were to be increased, which is the social cue, it would lead to an increase in the release of vasopressin, thus associating rewards with social signals, as observed by Guastella and their colleagues (Guastella et al., 2011). This would encourage the animal to engage in more social behaviour.

Studies have shown that rodents with irregular AVP levels were much more likely to exhibit

social impairments compared to their neurotypical counterparts (Carson et al., 2015). This has led to further studies to theorise and validate whether AVP can indeed be administered and used to enhance social and cognitive abilities in those with ASD.

Oztan et al. had shown that varied vasopressin concentration in the cerebrospinal fluid is a common trend in patients with ASD, as well as aggravating symptoms, compared to subjects who are healthy and ASD-free (Oztan et al., 2018). This was further confirmed by a 2018 study by Parker et al. which revealed that cerebrospinal fluid AVP concentration can be a "marker of sociality", and it provides the capacity to identify ASD cases (Parker et al., 2018).

The expression or activation of vasopressin V1a-receptor (V1aR) has also been demonstrated to be able to facilitate social and cooperative behaviour in voles (Donaldson et al., 2010) and mice, whether it be paternal behaviour, diminished aggressive tendencies, bond-forming, mating preferences etc. (Parker & Lee, 2001).

Vasopressin activity in the body is moderated by G-protein coupled-receptors (GPCRs) - AVPR1A (arginine vasopressin receptor 1-A, AVPR1B (arginine vasopressin receptor 1B), AVPR2 (arginine vasopressin receptor 2) and OXTR (oxytocin receptor). In terms of physiology, Thibonnier et al. point out that some of them are involved in regulating vasoconstriction, the volume of plasma, osmolality, thrombosis, corticotrophin release, etc. (Thibonnier et al., 2001). These receptors are also involved in the mediation of various behaviours, such as co-operative behaviour, anxiety or stress, aggressive tendencies, parental instincts, etc. (Baribeau & Anagnostou, 2015; Johnson & Young, 2017).

Therefore, vasopressin and its signalling pathway has been posited as a potential target in researching diagnostic criteria and developing drugs for ASD. However, despite this hypothesis, it is important to note that as of present, no direct implication of vasopressin has yet been clearly established in the pathophysiology of ASD.

3.1.1 Synthesis of Vasopressin

Pre-pro-vasopressin or AVP-neurophysin-copeptide, a pre-pro-hormone, is produced from the hypothalamic neurosecretory neurons. It is a signal peptide that is cleaved in the endoplasmic reticulum by a signalase to become pro-pressinphysin. Then in the Golgi apparatus, endopeptidase glycosylates the co-peptin and separates neurophysin and pro-AVP vasopressinyl-Gly-Lys-Arg peptide. Carboxypeptidase E trims this into vasopressinyl-Gly, which is then oxidised by glycine monooxygenase into vassopressinyl-hydroxyl-glycine). Lyase and glycolic acid then react with it to form the pro-configuration of arginine vasopressin (Ball et al., 2000).

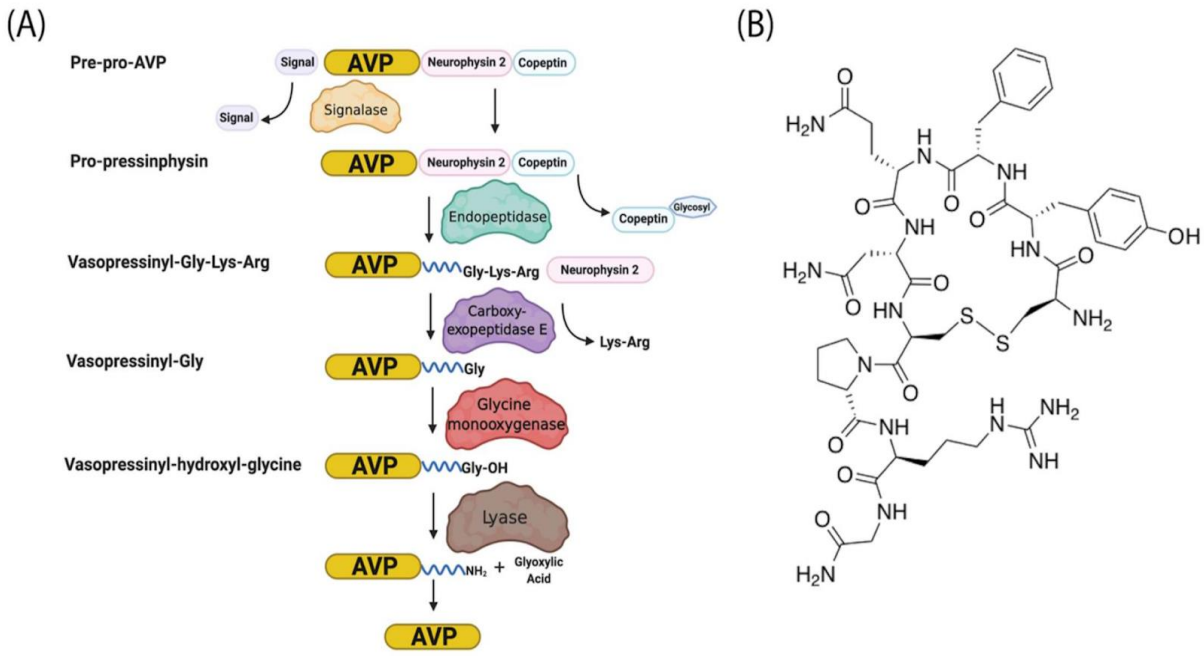

Fig 2: The route of synthesis and structure of arginine vasopressin (Sparapani et al., 2021).

This pro-AVP is reserved in the membrane-associated granules, until the activation of osmoreceptors and enhanced extracellular fluid osmolarity stimulates its release (Koshimizu et al., 2012), where it shapes into its mature form, circulating in the body as a freely flowing hormone for up to ten to thirty minutes of its release, until it finally faces enzymatic degradation

in the liver and kidney (Baumann & Dingman, 1976).

3.1.2 Mechanism of Action of Vasopressin

Arginine vasopressin assists the regulation of social and cooperative behaviour. Stored and acting from neural networks located in the amygdala, medial preoptic area, lateral septum, hypothalamus and periaqueductal grey (Johnson & Young, 2017; Moore & Lowry, 1998), it seems to exert specific responses which are possibly influenced by differences in distribution, neural AVP-receptor expression and gonadal hormones (Albers, 2015). Huber et al. recorded in a 2005 study that AVP enhances neural transmission and partakes in neuroendocrine stress response (Huber et al., 2005). AVP and oxytocin seem to have many overlapping characteristics, sharing the same seven of their nine amino acids in their structure, leading to their systems being intertwined, yet being able to function variably, and even antagonistically at times depending on context (Donaldson et al., 2010). Both of its receptors, V1aR and V1bR, express widely in regions of the brain.

Koshimizu et al. and other researchers have suggested the involvement of the V1aR in the mediation of parental behaviour, memory, pair-bonding, recognition of individuals, etc. (Albers, 2012; Koshimizu et al., 2012; Goodson & Bass, 2001). While its exact route of action remains unclear, microinjections of AVP or V1aR agonists to the lateral septum and overexpression of V1aR has exhibited improvement of social recognition, and contrarily, opposite studies using V1aR antagonist have shown to impair the same (Bielsky et al., 2004; Stemmelin et al., 2005), validating that V1aR indeed plays a significant role in mediating sociality in animals. Ahn et al. and Watkins et al. had further tested vasopressin receptor V1aR action on diffused expression and confirmed its ability of regulating adaptive and emotional behaviour as well as pain (Ahn et al., 2001; Watkins et al., 1986). Other than these, V1aR and vasopressin is known to play major roles in various regions of the brain, such as circadian

rhythm (by acting on the suprachiasmatic nucleus) (Li et al., 2009) and synthesis and secretion of cortisol (by acting on the hypothalamic-pituitary-adrenal axis) (Pasquali et al., 1999).

AVP in the anterior pituitary gland and adrenal medulla causes V1bR to express, promoting the release of adrenocorticotropic hormone (ACTH), which in turn elevates the cortisol release and V1aR-regulated synthesis process which is taking place in the adrenals (Pasquali et al., 1999; Shanik et al., 2008). This causes an endocrine response when the animal is in stress. Anomalous levels of this ACTH, and by conjunction knocked-out V1bR, is linked with impaired stress response, responsive aggression and social deficits (Bosch, 2013; Wersinger et al., 2007; Roper et al., 2011).

3.2 Intranasal delivery system

The intranasal delivery system is a route of administering drugs that is non-invasive and the drug can be delivered locally or systemically. The drug is sprayed into the nose cavity via nostrils, which adheres to the inside walls of the nose containing blood vessels, which in turn absorb the drug into the bloodstream, bypassing the first-pass metabolism process, cutting down on wastage of administered products, causing fewer side effects, reducing risks of syringe-related injuries, as well as being quite convenient to apply and having a very quick onset of action, as detailed by a Grassin-Delyle et al. study in 2012 (Grassin-Delyle et al., 2012).

Guastella et al. reported intranasal drug delivery to be quite convenient for and tolerated well by paediatric patients, for those diagnosed with ASD (Guastella et al., 2015) among other disorders, especially if the administration has to be long term. However, this route is not perfect. Drugs administered nasally can reach the central nervous system directly through the trigeminal and olfactory nerve fibres (Thorne et al., 2004) and indirectly through systemic circulation, the former being situated in the upper posterior area of the nasal cavity, which can

be hard to target and thus undermine the full potential of the drug. Quintana et al. note that there are many factors that can negatively affect drugs delivered via the intranasal route. Some of them are- physicochemical factors (stability, molecular weight and lipophilicity), limited consistency, control and accuracy due to physiology, reliable deposition of particles and bioavailability. The nose-to-brain route is difficult to target in this particular pathway due to many variables (Quintana et al., 2016).

3.3 Administration of Vasopressin

In healthy human volunteers, intranasal vasopressin has been shown to facilitate the proper interpretation of emotions via observing facial features (Guastella et al., 2010) in others as well as the understanding of appropriate social cues and behaviours.

In patients with diabetes insipidus, intranasal AVP can improve memory functions (Laczi et al., 1982). Again, in patients with post-stroke aphasia, intranasal AVP has been shown to optimise simple speech and composition capabilities (Tsikunov & Belokoskova, 2007).

Intranasal vasopressin has been demonstrated by Born et al. to bypass the bloodstream and directly act on the cerebrospinal fluid within 30 minutes (Born et al., 2002), causing an accumulation of peptides aka plasma vasopressin concentration in that region compared to subjects who were placebo-treated. This is useful for the AVP to target neural pathways and exert its therapeutic effects in cases of brain disorders and neurodivergent conditions, especially in males.

Moreover, Young et al. had illustrated the role of the central administration of vasopressin in the regulation of paternal behaviour and suppressing of aggressive tendencies in adult male voles directed at cubs, further providing evidence for AVP in the facilitation of appropriate social behaviour (Young et al., 1999).

Despite extensive documentation on the positive effects of vasopressin administration on social behaviours, until recently, intranasal AVP has not exclusively been tested for the treatment of ASD. However, electro-acupuncture or transcutaneous electrical acu-point stimulation (TEAS), a non-pharmaceutical approach, has been proven to facilitate the treatment of ASD. Zhang et al. have provided evidence of being able to enhance concentrations of AVP in the brain and thus exhibit their roles in improving social behaviours and symptoms of anxiety in rodents (Zhang et al., 2015) and autistic children (Zhang et al., 2012).

Again, desmopressin is a synthetic analogue of vasopressin and is a very popular medication that has been used for a very long time in treating children with bed-wetting habits or nocturnal enuresis (Walle et al., 2007), an action that is common in children with autism (Niemczyk et al., 2018). Desmopressin itself has not been proven to improve the characteristic symptoms of ASD, but perhaps this can be explained by the fact that it is administered before the child is put to sleep, and they are not exposed to social situations, it acting selectively on AVPR2 rather than AVPR1A (Robben et al., 2004), as well as the fact it is administered via the oral route, and oral desmopressin is incapable of crossing the blood-brain barrier (Sørensen et al., 1984).

3.4 Setting of the study

Investigational New Drug Application #118327 involved in the Parker et al. clinical trial (Parker et al., 2019) had been recorded with the USFDA and the Institutional Review Board of Stanford University had greenlit the study to be a collaborative work of Stanford University's Departments of Psychiatry and Behavioural Sciences, Paediatrics and Comparative Medicine at the Autism and Developmental Disorders Clinic and supervised by the Data Safety Monitoring Board.

A group of thirty children who had been screened and their previous ASD diagnoses confirmed by paediatric psychologists in accordance with the Diagnostic and Statistical Manual of Mental

Disorders, Autism Diagnostic Interview and Autism Diagnostic Observation Schedule were enlisted to participate in this study. Among them, 25 were male, 5 were female; 19 were Caucasians, 11 were of other races. None of the (male) participants had undergone puberty, as previously Delville et al. had demonstrated that testosterone and administered vasopressin can work in conjunction to promote aggressive tendencies in males (Delville et al., 1996).

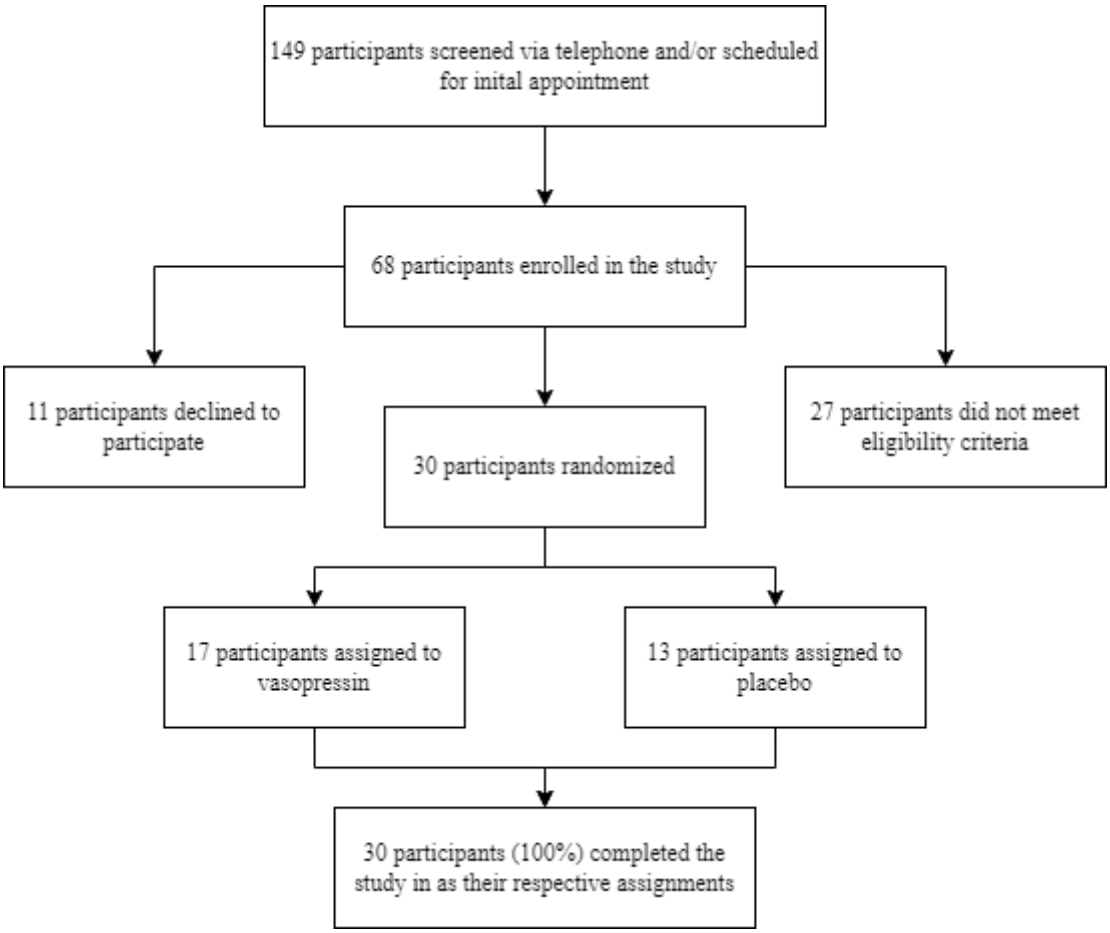

Fig 3: Flow diagram for phase 2 clinical trial and screening of the participants (Parker et al. 2019).

After successful enlistment, written consent had been obtained from their legal guardians and then the participants had been subjected to a baseline or pre-treatment measure test which would allow for comparative quantification later on. This had included assessing the participants' behavioural symptoms, capabilities, as well as taking their blood samples, tolerability levels, serum vasopressin concentrations and receptor genes using standard

protocols and common kits available in the market. This had been done one to four weeks before the drug would be administered.

Pre-treatment measure test	AVP	Placebo
SRS-2 T scores	78.12±1.66	83.00±1.90
CGI-S scores	4.82±0.15	4.77±0.17

Table 1: Pre-treatment or baseline scores for placebo and AVP-treated groups (Parker et al., 2019).

A double-blind, randomised and parallel design had been employed alongside the use of placebos which allowed proper evaluation of the tolerability and efficacy of the intranasal-administered drug product on the subjects. The randomised design, with the assignment of treatment aided by a schedule generated by a computer, disallowed participating children, their guardians and the research group from explicitly knowing whether the product the subject was assigned to a specific participant was a placebo or the real drug. This ensured that the results were free of bias and remained uncompromised. The team had evaluated the parents on their ability to pinpoint the conditions of the treatment that their youngster was given and had concluded that they were unable to, confirming the success of eliminating biassed opinions. The testing period spanned over four weeks, during which injectable, sterile vasopressin and placebo solution (having the same composition as the former, except the main active ingredient) had been acquired and divided into 20 IU (international unit) or 25 ml sterile amber-glass bottles fitted with metered nasal spray applicators. The containers and applicators for the drug product were also made to be essentially indistinguishable from the ones provided for the placebo, their exact contents only being known to the pharmacists involved in their preparation,

further eliminating all possibilities of variables relating to treatment conditions to affect the outcome of the test.

A dose-amplification style of the regimen had been followed, with 4 IU being administered to the subjects in the 1st week, 8 IU in the 2nd, 12 IU in the 3rd and 12 IU in the 4th (for 6 to 9.5-year-olds) or 16 IU in the 4th (for those above 9.5 years). After administration, on a weekly basis, the participants' vital signs, height-weight, electrocardiogram and clinical tests had been performed to monitor the tolerability and safety of the drug. On top of that, at home, guardians had been instructed to note down observations regarding their children's behaviours or any changes they might notice. Care was taken to properly educate and train the participants' legal guardians/caregivers so that they would be able to properly evaluate their children and note down their improvements or declines.

After the testing period had concluded, the participants' vitals, tolerability, baseline values and blood samples were retaken, as well as having to undergo several behavioural examinations, such as Reading the Mind in the Eyes Test (RMET), Developmental Neuropsychological Assessment (NEPSY), Theory of Mind, Facial Expression Recognition Test (FERT) and Social Perception Domain tests for Affective Recognition.

3.5 Findings of the studies

As it is known, the administration of intranasal vasopressin leads to the increase of vasopressin levels in the blood and cerebrospinal fluid (Born et al., 2002), and this implies the given treatment would have an effect on the physiological and behavioural processes regulated by vasopressin in the human body.

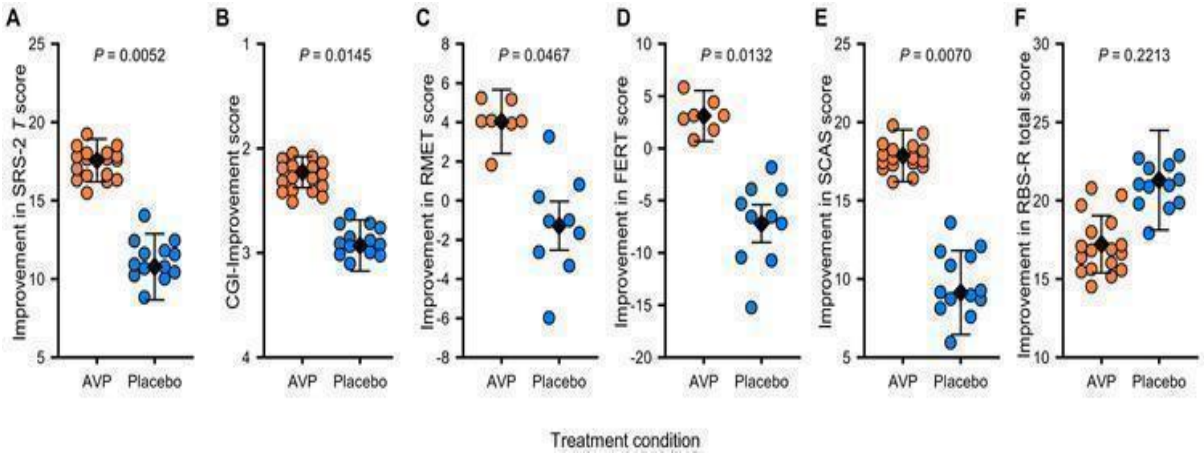

Fig 4: Graphs showcasing and comparing the improvement in scores between the AVP and placebo-treated groups via various primary and secondary outcome measure tests (Parker et al., 2019).

This had been confirmed as the participants who were administered with intranasal vasopressin showed greater amelioration when compared to those who were given only a placebo, and this kind of improvement was especially significant and noticeable in the participants who had been tested with a higher concentration of pre-treatment serum AVP.

3.6 Results of outcome measure tests

Arginine vasopressin, which was administered through the intranasal route, had successfully treated the core symptoms of ASD, unlike previously available drug therapies, as can be observed via various outcome measure tests noted below. It had improved social, communication and motor skills, as well as giving the scope of better control over anxiety symptoms. Not only was the drug tolerated well and safely in the young participants, no significant variations in vital values or adverse events had transpired. The mode of administration employed was convenient, non-invasive and tolerable for the target age group. It also allowed for rapid absorption into the bloodstream and onset of action in the cerebrospinal

fluid, and there was minimal wastage of drug substances due to bypassing the first-pass metabolism.

3.6.1 Primary outcome measure tests

In order to assess the participants and the outcome of the employed therapy, a primary outcome measure had been applied. The Social Responsiveness Scale-2 test (SRS-2 T) as well as its subscale, the Social Communication and Interaction (SCI) were utilised as the primary outcome measure. Various probes by Constantino and others provided evidence on the fact that these scores are useful in deducing the severity of the social deficits caused by conditions such as ASD (Constantino & Todd, 2003; Constantino, 2011; Constantino & Todd, 2005). The legitimacy is validated by the fact that it corresponds to the scores of DSM (Constantino et al., 2003) as well as previous studies (Yatawara et al., 2016). Here, it had helped exhibit that the intranasal AVP-treated participants indeed had improved social and perception skills and higher test scores than those being given the placebo, eliciting a more significant response to the treatment administered. Participants administered with AVP had shown an improved score of 17.6±1.37 on the SRS-2 T-scale with 17.5±1.37 on the SCI-scale compared to 10.8±2.11 and 9.75±2.11 for those with the placebo respectively.

3.6.2 Secondary outcome measure tests

In regards to the secondary outcome measure, the Clinical Global Impression-Improvement scale (CGI-S) test had been utilised to evaluate the participants' potential degree of improvement to their social, cognitive and communication skills, as well as in non-core domains (Guy, 1976). This displayed an improved score of 0.873±0.126 for those who were given AVP compared to 0.712±0.202 for the placebo group. Several other tests were also employed by specialists, such as the Reading the Mind in the Eyes test (RMET) to evaluate the participants' capability of understanding the emotions displayed by other individuals, and the

Facial Emotion Recognition test (FERT) to evaluate the participants' capability of perceiving different facial emotions. Again, participants treated with AVP, and especially those with higher serum AVP levels during pre-treatment or baseline analyses, performed better than the control group. Moreover, the legitimacy of the response by the AVP-treated group was further validated by the participants' caregivers. The AVP-treated group improved their score on RMET by 4.04±1.63, while for the placebo-treated, it was −1.28±1.24, and for FERT, the numbers were 3.10±2.42 and −7.19±1.81 respectively.

Outcome measure test	**Score improvements**			
	AVP	Placebo	AVP	Placebo
Primary outcome measure scores				
SRS-2 T scores	17.6±1.37	10.8±2.11	More	Less
SCI	17.5±1.37	9.75±2.11	More	Less
RBR	15.6±1.73	12.7±2.70	More	n/s
Secondary outcome measure (clinician-evaluation)				
CGI-S	0.873±0.126	0.712±0.202	-	-
CGI-I	2.23±0.149	2.93±0.244	More	n/s
Secondary outcome measure (guardian-evaluation)				

SCAS	17.9±1.66	9.14±2.68	More	Less
RBS-R	17.2±1.83	21.3±3.18	More	Less
Secondary outcome measure (performance of the child)				
FERT	3.10±2.42	−7.19±1.81	-	-
RMET	4.04±1.63	−1.28±1.24	-	-
NEPSY (Theory of mind)	2.1±1.41	0.677±1.20	-	-
NEPSY (Affect recognition)	0.094±2.20	−2.58±1.90	-	-

Table 2: Post-treatment scores for placebo and AVP-treated groups (Parker et al., 2019).

3.7 Safety considerations

Intranasal vasopressin was administered to a group of 17 children and had displayed no remarkable adverse reaction event or remarkable variations in the participants' vital signs, ECG, clinical or physiological values.

In the case of the AVP-treated group, 24% had reported agitation, insomnia and decreased appetite individually, 18% nasal congestion and 12% fever, headache and nausea. However, these percentages were not very far off from the placebo-treated groups, comprised of 13 participants, who more or less reported similar events at a similar rate of occurrence.

Thus, it was confirmed that administration of intranasal vasopressin had been tolerated well by the participants throughout the 4-week time period of therapy.

3.8 Contraindications

It can be deduced that individuals with higher serum vasopressin levels responded better to the treatment. This validates the theory that pre-treatment concentrations of certain neuropeptides (in this case, serum vasopressin) in blood could help in the prediction of the response to the given treatment. However, this also could have proven to contraindicate previous studies where it was shown that low vasopressin levels in the cerebrospinal fluid was a definitive marker of ASD-affected individuals (Thibonnier et al., 2001; Robben et al., 2004) and this could have meant that among the participants, the ones with lower endogenous serum vasopressin concentrations should have been the one to benefit the most from the applied treatment, rather than the other way around, as it has been observed. However, there are still debates on the exact relation between vasopressin concentrations in the blood and its activity on the brain (Donaldson & Young, 2008; Carson et al., 2014; Kagerbauer et al., 2013; Wotjak et al., 1998). There is also the possibility that participants with lower serum AVP were under-dosed, as Parker et al.'s pilot study only utilised a very modest dose increase in terms of the dose regimen.

There is also a possibility of the body's tendency of developing resistance to the neuropeptide in question. Similar to Type-2 diabetes mellitus, which is marked by the body having a lowered sensitivity to insulin, and needing to be on insulin therapy, yet the body manifests as having a high serum insulin concentration as its tendency for compensation, as found in a 2008 paper by Shanik et al. (Shanik et al., 2008). In a similar fashion, the participants who have been shown to have high serum vasopressin concentrations during pre-treatment tests could have some form of lowered sensitivity to their own body's AVP, and yet they are the same group excelling with the administration of intranasal AVP.

Nevertheless, vasopressin agonists are not the only "novel" treatment at present being developed to treat ASD. Vasopressin receptor antagonists are also currently being tested for

their efficiency. This logic of focusing on vasopressin receptor antagonists rather than vasopressin agonists is not unfounded, being based on the fact that administration of vasopressin has been shown to promote aggressive tendencies of rodent subjects (Ferris et al., 1997; Gobrogge et al., 2009), as well as the human subjects (Thompson et al., 2004), especially the males. Umbricht et al. had reported some improvements, such as enhanced visual, behavioural, cognitive, emotional and social functions, after administering RG7713, a small molecule vasopressin receptor AVPR1A antagonist, to adults with high-functioning ASD (males aged between 18 to 45 years) (Umbricht et al., 2017). The most promising study yet was on Balovaptan.

Index	**Parker et al. study**	**Bolognani et al. study**
Utilised molecule	Arginine vasopressin.	Balovaptan.
Mechanism of action	V1a, V1b-receptor agonism.	V1a-receptor antagonism.
Route of administration	Intranasal.	Oral.
Participants	Children (6 to 13 years).	Adult men (22 to 26 years).
Primary outcome measure results	Large effect size.	Null.
Secondary outcome measure results	Improved social, motor functions, anxiolytics.	Improved adaptive behaviours.

Table 3: Comparison of the Parker et al. and Bolognani et al. clinical trials (Forgeot et al., 2019).

Bolognani et al. had developed and tested Balovaptan (RG7314), a notable vasopressin receptor antagonist whose secondary outcome measure, having utilised the Vineland-II Adaptive Behaviour Scale, led caregivers and clinicians to conclude that the treatment strategy had a positive influence on the participants, despite observing no remarkable results on the primary outcome measure tests (Bolognani et al., 2019). This particular drug has recently been granted the Breakthrough Therapy Designation by the US Food and Drug Administration (FDA) for treating individuals with ASD in 2018 as reported in Roche (Roche, 2018).

However, it must also be mentioned that these findings were based on studies that were performed on neurotypical subjects, who did not have an impaired internal vasopressin system, an evident marker in individuals, whether human or animal, with ASD. Moreover, vasopressin agonists have elicited a better response from children and it is being assumed that this is taking place due to being influenced by childhood development processes. Parker et al.'s participants being children, individuals who are still capable of neuroplasticity, which is the ability of the brains of young animals to modify and adapt due to their young, developmental age, might grant credibility to vasopressin agonists performing better in children.

Chapter 4: Conclusion

The purpose of this write-up was to review studies and clinical trials that tested the efficiency of intranasal vasopressin in the treatment of participating paediatric patients who have been diagnosed with and was being medicated for autism spectrum disorder (ASD), a debilitating neurodevelopmental disorder marked by social, communication, intellectual deficits and sensory overload, at the time of the study. This is grounded on previously done research that had successfully demonstrated the neuropeptide arginine vasopressin being a sturdy biomarker that pointed to the occurrence of ASD, as well as playing vital roles in the promotion of appropriate social functioning in mammals.

The Parker et al. trial had taken place to study how the administration of intranasal vasopressin boosts social and communication functioning and suppresses anxiety and repetitive behavioural symptoms in children with autism spectrum disorder compared to a control group treated with placebo generated a favourable result. Moreover, the participants recorded having higher pre-treatment serum AVP levels had reaped the most benefits from the treatment, implying that this aspect can be utilised in the future to establish guidelines for dosing. Intranasal AVP also was observed to be tolerated well by the participants with no significant adverse event transpiring. The entire duration of intranasal AVP administration did not disrupt the normal vital signs, body weight-height values, clinical-chemical values or electrocardiogram tracings in the participants.

4.1 Limitations

The sample used in most of the studies consisted of a smaller number of participants. And while the reports and trials were an overall success, it would not be wrong to suggest that the topic of the study could have suffered from the lower sample size as well as biassed sampling. Not

only do the participants have ASD, a condition that has several aetiologies, but the majority of the participants were also male. The participants were on their respective prescribed medications and it was ascertained that none of their medications actually interacted with the administered intranasal vasopressin. The primary and secondary measures of outcome in Parker et al.'s trial (Parker et al., 2019) depended on reporting of behavioural changes by the parents or caregivers, a practice which will remain subjective no matter how efficient the benchmarks the instruments for reporting are.

There was significant trouble encountered while searching and trying to access several cross-reference articles being put behind expensive paywalls, an experience which can prove to be detrimental in the long run as it puts a monopoly on scientific knowledge, and can hamper the scopes and capabilities of research.

The lack of extensive literature specifically on intranasal vasopressin for ASD treatment was also a problem, however, this topic being in its infancy should be a point of encouragement for future researchers, such as myself, to design my own research model and plan regarding this particular topic.

4.2 Future prospects

In the future, if it is possible to utilise a larger sample size and bigger clinical trials, it would certainly help to ensure some aspects- the evaluation of endogenous vasopressin concentrations, its relation to the highest tolerable dose of AVP and outcome measures associated with brain activity. It would help identify the importance of serum AVP concentration in setting dose regimens and the biological significance of the neuropeptide itself. It would also be possible to conduct tests on a wider variety of subjects who perhaps do not fit the present inclusion criteria mentioned in this particular study, which had excluded those with cardiac, hepatic and renal disorders, seizures, etc. Moreover, in the interest of the

safety of the young participants, a very short trial period was undertaken. In the future, this particular study can surely benefit from a wider investigation centred on preclinical, safety and toxicological values that will allow young participants to safely partake in the drug treatment trial for a longer time period and more specified results.

Another scope of research that should be investigated is the integration of drug therapy with behavioural interventions. The combination approach would allow the research team to establish methods on how to better treat and control ASD symptoms. Grzadzinski et al. had exhibited that the method of observing changes in social communication can be utilised to identify the clinical changes that are occurring due to the treatment, and it has proven to be a very sensitive mode of testing patients with ASD (Grzadzinski et al., 2016).

Arginine vasopressin and its administration to children with ASD was a treatment method strategized and conducted by compiling previously known data on the effects of vasopressin and its effects on individuals with ASD. This has presumably opened a new door in the treatment of autism and future research delving into the specifics on causations and larger sample sizes will result in confirmation of found data. Widening the scope of research by applying a definite research model to a wider range of participants still remains a point of further investigation, as this particular study did exclude certain individuals who did not fully fit the research team's mentioned inclusion criteria. ASD itself does not have a specific list of criteria that it fulfils, as it is a very diverse condition, and this exclusion step was simply taken as a precautionary step so as not to overwhelm or endanger those with heavy ASD symptoms.

To sum it up, intranasal vasopressin has proven its potential for treating patients with autism spectrum disorder, especially their social symptoms, and holds great promise in the future for the treatment of ASD.

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