CHAPTER 2

LITERATURE REVIEW

"A literature review is not simply a catalogue of annotated summaries but an intellectual effort to understand the state of art in a particular area.''

-Michael Connelly

2. Literature review

2.1 Peptide therapeutics

Peptides are unique therapeutic agents that are used to treat a number of severe and incurable diseases. Peptide therapies are tissue-selective, biocompatible, capable of being scaled up economically, and capable of mitigating many of the drawbacks of small molecule medications. Since insulin was discovered in 1921 and approved for medical use in 1982 [1], therapeutic peptides had ongoing success stories of treating many diseases, including diabetes, inflammatory diseases, cancer, etc [2]. Compared to traditional small molecules, they show low side effects, have a great safety profile, and are very selective and strong [1]. The USFDA (United State Food and Drug Administration) has approved several biomacromolecules for their clinical use up to now [3]. The clinical applications of peptide therapies are, however, limited by unfavourable attributes such as rapid metabolism, rapid decomposition in plasma, negative-charge density, and traditional delivery methods that result in inadequate patient compliance. The potential for tailored delivery vehicles that can selectively transport and offer better stability of loaded peptides from the biological environment will determine the eventual outcome of peptide-based treatments. Recent developments in polymeric and nanoengineered lipid-based delivery methods have expanded the range of peptide's medicinal targets.

The identification of possible therapeutic peptides is a key component of peptide development; hence, their synthesis and sequence modification follow to improve their pharmacological effects. The biological activity of peptides is determined by their chemical structure mostly related to their intended therapeutic effect [4]. The development of peptide drugs necessitates application of medicinal chemistry techniques to enhance their properties, allowing them to mimic, stabilize, or create a model of secondary or tertiary structures after they have been synthesized. This modification process is essential for broadening their biological activity and achieving characteristics such as specificity, stability, and solubility. A range of innovative methods is employed to produce stabilized peptides and proteins. These methods include chemical synthesis, where the peptides are carefully constructed, and chemical modifications that alter their structure. Additionally, the integration of omics technologies, adjustments made

to either side chains or backbone of peptides, and the process of cyclization are also utilized. Various strategies such as mimicking the structures of α-helices and enhancing their stability are pivotal, as are the imitation of β-strands and sheets. Furthermore, the use of recombinant technology allows for the efficient production of peptides, and PEGylation enhances the properties of both peptides and proteins. Notably, peptide modification can also occur through the expansion of the genetic code, leading to novel functionalities. Lastly, advancements in the delivery systems for peptide medications are being explored, focusing on covalent interactions that facilitate effective transport and function of these therapeutic agents [5]. The most recent and effective technique for creating therapeutic peptide analogues with the intended and targeted structures is chemical modification. The main impacts of peptide modification are increased stability, selectivity, and activity. The three finest examples of the results of chemical modification techniques that are currently being used in clinical applications are semaglutide, liraglutide, and selepressin. However, certain chemical modifications are unable to simultaneously recover the proteolytic stability, selectivity, and activity.

For example, peptide half-life can typically be extended by substituting L-amino acid with D-amino acid. However, D-amino acid- modified peptides modification rarely demonstrate biological activity efficiently [6]. Peptide modifications enable them to exhibit improved plasma stability and activity, as well as to become more drug-like [7].

2.2 Challenges of peptides as therapeutic agents

Peptide-based treatment prospects are increasingly showing their immense potential in the stubborn illnesses management due to their higher biological activity, higher selectivity, lower toxicity. However, due to several unavoidable limitations, such as target selectivity, intracellular activity, and stability, the therapeutic applications of these peptides may be more robust (Figure 2.1). Furthermore, because of their structural complexity, peptides, proteins, and nucleic acids are still difficult to provide therapeutically.

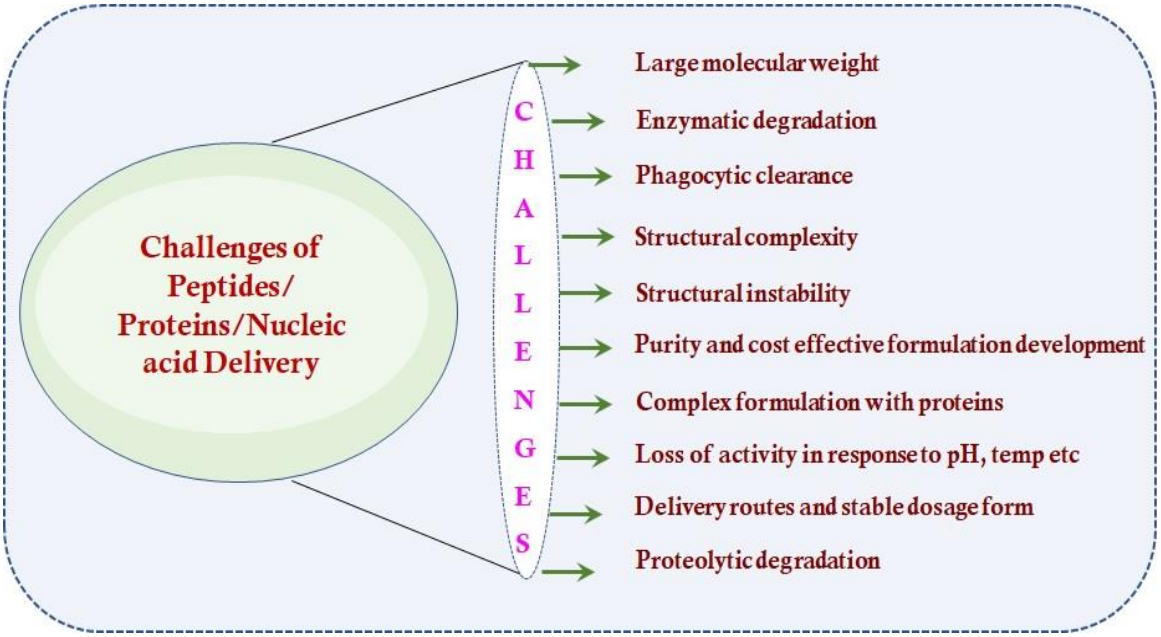

Figure 2.1: Various challenges of delivering macromolecules

The effectiveness of oral administration of these macromolecules is hindered by a number of physiological and biological conditions, including the presence of ingested food, enzyme degradation, the mucus layer, gastrointestinal system acidic pH, low oral bioavailability of peptides/proteins, etc. Furthermore, peptidases quickly break down peptides and tiny proteins that are poorly absorbed by mucosal membranes, resulting in a short half-life in circulation. Consequently, the intravenous, intramuscular, or subcutaneous methods are used to administer the majority of peptide/proteins/NA-based formulations [8]. Systemic distribution of proteins, peptides, and nucleic acids is hindered by a number of issues, such as poor intracellular trafficking, insufficient cellular affinity, and short half-lives due to destruction by cellular nucleases and plasma [9]. Moreover, even with parenteral delivery, metalloproteinase-mediated enzymatic breakdown, plasma instability, quick renal clearance, and potential immune system reactions are still unavoidable [10].

These elements taken together produce the small half-life of these molecules, which causes repeated dosing needed to reach plasma level at steady state, hence lowering patient compliance and limiting their clinical usefulness. Therefore, ready to use formulations for selected peptide molecules are indeed needed which can infuse the therapeutic agent at slower rate and would able to uphold the adequate plasma concentration of the drug.

The complexity of peptide administration that is selective, requiring navigating multiple biological hurdles prior to realizing the full potential of peptide treatment. Additionally, adverse traits such as quick deterioration in negative-charge density, quick metabolism, plasma, and traditional delivery approaches that result in low patient compliance, limit the peptide's clinical applications treatment methods. To render these interactions ineffective, strategies utilizing recombinant engineering, hydrophobic polymer-based chemical modification, and micro- and nanoencapsulation based Peptide delivery shows enormous potential and has been widely investigated in the previous fifteen years of study [11]. These findings protect the peptide medicines from being broken down by enzymes, stop the first explosion and uncontrolled release, and raise plasma half-life, lower the frequency of doses, increase patient adherence, and selectively deliver the bioactive, with few negative effects and consistently remain effective against chronic diseases [12].

2.3 Drug profile

2.3.1 Oxytocin

Comprising nine amino acids, the nonapeptide oxytocin (OXT) is synthesised by the mammalian brain's hypothalamus and acts as a neuropeptide hormone. In women, it is mostly used for parturition management, postpartum uterine bleeding control, treatment for inevitable, incomplete, voluntary abortion, lactation, etc. [1]. OXT functions as both a neuromodulator and a neurotransmitter in the brain. In preclinical research, peptides linked to neurotransmitters demonstrated remarkable efficacy in treating conditions related to the brain and were identified potential latent therapies in neurological illnesses.

Among the several neuropeptides, OXT serves as a neuroprotective by shielding the hippocampal from brain cells excitotoxicity and as a chemical transmitter, also reducing inflammation in neurones [13].

2.3.1.1 Functions of OXT

OXT is derived from the Greek word “oxutokia” meaning “quick birth” and has been generally known as “love hormone”. With the arena of information broadening progressively (Box 1) [14]. Based upon the multiple functions that this hormone carries out, two main systems have been recognized; the peripheral OXT system that reflects secretion from the pituitary gland

and the central OXT system. The behavioural consequences of this hormone are hypothesised to reflect OXT secretion from centrally projecting neurones different from those entering the posterior pituitary or which are collaterals from them [14]. This belief is mostly due to the fact that OXT once secreted from the pituitary gland cannot re-enter the brain owing to the BBB [15].

BOX 1: Functions of Oxytocin

Peripheral action	Central action
• Let-down reflex • Penile erection and ejaculation • Parturition and uterine involution • Modulation of testicular steroidogenesis • Cellular proliferation, • Reduces the secretion of urine • Stimulates secretion of sodium from kidneys (natriuresis) • It may play a role in autism	• Improves sexual behaviour, • Improves social behaviour, • Improves maternal behaviour, • Improves lactation and grooming • Tolerance and dependence to opioids

2.3.1.2 Pharmacology and Biochemistry

Mechanism of action of Oxytocin

A polypeptide characterized by its seven transmembrane domains, OXT belongs to the Rhodopsin-type class-I G-protein coupled receptor (GPCR) family. It exhibits a notable affinity for a specific group of proteins situated on the surface of target cells, which are classified as OXT-receptors [16].These OXT-receptors are linked to Gq subtype G-proteins, which, upon binding with GTP, significantly increase the activity of the enzyme known as Phospholipase-C [17]. When OXT fixes to its receptor, it commences a series of cellular events leading to the activation of the Phospholipase-C enzyme [18]. This enzyme plays a crucial role in accelerating the production of inositol triphosphate and 1,2-diacylglycerol, two important signaling molecules. The inositol triphosphate produced triggers the release of intracellular calcium ions (Ca^{++}), setting off a series of vital processes within the cell.

As calcium ions bind to the protein Calmodulin, they form a complex known as the Ca^{++}-Calmodulin complex. This interaction is particularly significant in smooth muscle tissues, like those found in the myometrium, where the complex then triggers the MLCK (Myosin Light

Chain Kinase) enzyme. The activation of MLCK is a key step that leads to the contraction of smooth muscle fibers [19]. Additionally, one of the noteworthy secondary effects of OXT is its ability to enhance the synthesis of prostaglandin F2α (PGF2α), further influencing muscle activity and other physiological responses [20].

Pharmacokinetics of oxytocin

- **Absorption**

Oxytocin is given through an IV and is fully soluble. After intravenous treatment, it takes about 40 min for oxytocin levels in the plasma to reach a steady state.

- **Distribution**

Oxytocin is spread out in the fluid outside of cells. Oxytocin probably gets into the bloodstream of the foetus in small amounts.

- **Metabolism**

Only a little amount of the neurohormone is eliminated in the urine unaltered; the enzyme oxytocinase is mostly in charge of controlling oxytocin levels in pregnancy. Throughout pregnancy, oxytocinase activity rises; near term in the plasma, placenta, and uterus it peaks. During gestation, the placenta is a major source of oxytocinase and generates rising enzyme amounts in reaction to rising oxytocin levels generated by the mother. Mammary glands, the heart, the kidneys, and the small intestine all show oxytocinase activity too. The brain, liver, spleen, skeletal muscle, colon, testes all show lower degrees of activity. Men, cord blood, and non-pregnant women all have very low degrees of oxytocin breakdown.

- **Excretion**

The liver and kidney help mostly to explain its quick disappearance from plasma. The urine contains just little levels of oxytocin, unaltered.

2.3.1.3 Clinical usage of OXT in Gynaecology and obstetrics: a life-saving drug in women

The World Health Organization (WHO) reports that complications during pregnancy and childbirth claim the lives of 500,000 women annually in Latin America, Asia, and Africa. At least 25% of those fatalities are related to postpartum hemorrhage, which is mostly caused by the uterus's failure to contract sufficiently following delivery (atonicity).

The preferred drug to prevent post-partum haemorrhage is OXT [21]. Because of the characteristic lifesaving properties of OXT, it was argued that OXT be listed as an essential medicine in the National List of Essential Medicines (NLEM), in 2015. Further, in March 2017 in the 20th WHO, OXT was confirmed as an *essential medicine* and included in the Model List of Essential Medicines. The country's largest group representing doctors, the Indian Medical Association (IMA), said that oxytocin's ability to prevent haemorrhage after birth meant it was "not a drug that doctors can compromise on" [22]. However, due to the Government of India ban, imposed on OXT, the legal status of OXT, a medication crucial for the health and well-being of new mothers is still in the halfway house, which will be discussed in subsequent sections [23]. In gynaecology and obstetrics, the dose, applications and routes of administration of OXT are given in table 2.1. Currently, many OXT products in various regulatory markets across the world are available which are summarized in table 2.2 along with their application.

Table 2.1: Dose of OXT in human

Particulars	**(Oxytocin injection I.P.)**		
Strength/Packing	5 units/0.5mL/Ampoule & 5 units/mL/Ampoule		
	During labor induction	Control of postpartum hemorrhage	Treatment of incomplete, inevitable, or elective abortion
Administration	0.9% sodium chloride or Ringer's lactate (5/10 units OXT in 500/1000 ml dilution) by i.v	If the patient has i.v. infusion running, 10 - 40 units in electrolyte or dextrose solution remaining (depending on the amount of diluent remaining and max 40 units in 1000 mL) Or 10 units can be administered by i.m. after the delivery of the placenta	0.9% aqueous sodium chloride or 5% Dextrose (10 IU OXT in 500 ml dilution) by i.v.
Maximum dose	10 units	40 units	10 units
OXT/mL (during administration)	0.005 units/mL	0.04 units/mL	0.02 units/mL
Source: Pitocin IP pack insert [24]			

Table 2.2: Dose & uses of different OXT injections in human in various regulatory markets

Regulatory Market	Particulars	Diluents	During labour induction	Control of postpartum	Treatment of incomplete or inevitable abortion
US	West-Ward Pharmaceutical Corp	Normal saline (NS) or 5% Dextrose	i.v. infusion, 1 mL (10 units) is diluted with 1L of a diluent.	10 - 40 units of OXT diluted to 1L of a diluent and run at a rate required to control uterine atony. i.m. Administration 1 mL (10 units) of OXT can be administered post-delivery of the placenta	An intravenous infusion of 500 mL physiological saline solution or 5% dextrose in physiological saline, with 10 units of OXT added, should be administered.
	Fresenius Kabi USA	NS or 5% Dextrose	i.v. infusion, 1 mL (10 units) is diluted with 1L of a diluent.	10 - 40 units of OXT diluted to 1L of a diluent and run at a rate required to control uterine atony. i.m. Administration 1 mL (10 units) of OXT can be administered post-delivery of the placenta	An intravenous infusion of 500 mL physiological saline solution or 5% dextrose in physiological saline, with 10 units of OXT added, should be administered at a rate of 20 - 40 drops per minute.
	Par Pharmaceutical, Inc. (Pitocin®)	NS / 5% Dextrose/ Ringer's lactate	i.v. infusion by one mL (10 IU OXT) in 1L dilution (0.9% sodium chloride or Ringer's lactate)	10- 40 USP in dextrose or electrolyte solution lasting (max 40 Units in 1L) i.m. administration. 1 mL (10 units) can be given after the delivery of the placenta	10 IU OXT in 500 mL dilution (0.9% aqueous sodium chloride or 5% Dextrose)
	APP Pharmaceuticals, LLC	NS or 5% Dextrose	i.v. infusion, 1 mL (10 units) is diluted with 1L of a diluent.	10 - 40 units of OXT diluted to 1L of a diluent and run at a rate required to control uterine atony. i.m. Administration 1 mL (10 units) of OXT can be administered post-delivery of the placenta	An intravenous infusion of 500 mL physiological saline solution or 5% dextrose in physiological saline, with 10 units of OXT added, should be administered.
EMA	Aguettant Ltd	NS or 5% Dextrose	I.V. Infusion by 5 IU of OXT be mixed with 0.5L of a physiological	5 IU gradually iv., in severe cases by iv infusion of a solution comprising 5 to 20 IU of OXT in 500 mL diluent.	Administer 5 IU slowly via IV, followed in severe cases by an IV infusion of a solution containing 5

Regulatory Market	Particulars	Diluents	During labour induction	Control of postpartum	Treatment of incomplete or inevitable abortion
			electrolyte solution or 5% dextrose	Caesarean section: 5 IU by slow iv injection instantly subsequently delivery	to 20 IU of OXT in 500 mL of diluent, adjusted to a rate sufficient to manage uterine atony
	Syntocinon, Mylan Products Ltd.,	NS or 5% Dextrose	5 IU to be mixed with 500ml diluent	Caesarean section & prevention of postpartum uterine haemorrhage: 5 IU by i.v. infusion. Treatment of postpartum uterine haemorrhage: Administer 5 IU in 500 mL of diluent via IV infusion, followed in severe cases by an IV infusion of a solution containing 5 to 20 IU of OXT in 500 mL of diluent	5 IU in 500 mL by i.v. infusion
	Pan Pharma UK Ltd	NS or 5% Dextrose	The standard dose is 5 IU administered via IV infusion, mixed in 500 mL of a physiological electrolyte solution	5 IU by i.v. infusion (5 IU mixed with physiological electrolyte solution)	5 IU by i.v. infusion (5 IU mixed in 500 mL) diluent, if necessary, followed at a rate of 20 to 40 milliunits per minute.
	EVER Neuro Pharma GmbH, Austria	NS or 5% Dextrose	Add 5 IU of OXT to 500 mL of a physiological electrolyte solution. For patients who must avoid sodium chloride infusion, a 5% dextrose solution can be used as an alternative diluent	Caesarean section: Administer 5 IU via slow IV injection immediately after delivery Prevention of postpartum uterine haemorrhage: The usual dose is 5 IU gradually iv post-delivery of the placentas. Treatment of postpartum uterine haemorrhage: Administer 5 IU slowly via IV, followed in severe cases by an IV infusion of a solution containing 5 to 20 IU of OXT in 500 mL of diluent, infused at a rate required to control uterine atony.	5 IU gradually iv, if needed followed by iv infusion at a rate of 20 to 40 mU per min or higher

Regulatory Market	Particulars	Diluents	During labour induction	Control of postpartum	Treatment of incomplete or inevitable abortion
	Hameln pharmaceuticals ltd	NS or 5% Dextrose	Add 5 IU of OXT to 500 mL of normal saline (NS). For patients who need to avoid sodium chloride infusion, a 5% dextrose solution can be used as the diluent	Caesarean section: Administer 5 IU via IV infusion, with 5 IU diluted in a physiological electrolyte solution and given as an IV drip infusion Prevention of postpartum uterine haemorrhage: 5 IU by i.v. infusion (5 IU diluted 500 diluent)	Administer 5 IU via IV infusion, with 5 IU diluted in a physiological electrolyte solution and delivered as an IV drip infusion
TGA	Apotex Pty Ltd	NS or 5% Dextrose	10 IU OXT per 1 litre infusion fluid is recommended.	5-10 IU by i.m. injection or 5 IU by slow bolus i.v. injection. In patients given OXT by drip to induce or stimulate labour, the infusion should be continued during the third stage. Caesarean Section: 5 IU by i.v. infusion or slow bolus i.v. injection after delivery of the foetus	5-10 IU by i.m. injection or 5 IU by slow bolus i.v. injection
	Generic Health Pty Ltd	NS or 5% Dextrose	5-10 IU by i.m. injection or 5 IU by slow bolus i.v. injection. (10 IU OXT per 1 litre infusion fluid is recommended.)	Caesarean Section: 5 IU by i.v. infusion or slow bolus i.v. injection after delivery of the foetus	Not Available
	Rympa Injection OXT/ergometrine maleate 1mL injection ampoule	NA	• Active management of the third stage of labour. • Prevention and treatment of post-partum haemorrhage associated with uterine atony		

Regulatory Market	Particulars	Diluents	During labour induction	Control of postpartum	Treatment of incomplete or inevitable abortion
	Syntometrin ® Phebra2 Pty Ltd (OXT 5 IU/mL and ergometrine maleate 500 microgram/ mL)	NA	Active management of third stage of labour: 1 mL i.m. following delivery of the anterior shoulder, or immediately after delivery of the child	Prevention and treatment of post-partum haemorrhage: Administer 1 mL intramuscularly following the expulsion of the placenta, or if bleeding occurs. If necessary, the 1 mL injection may be repeated after an interval of at least two hours. The total dose within 24 hours should not exceed 3 mL.	Not given
Canada	Fresenius Kabi Canada Ltd	NS, NS in 5% Dextrose	i.v infusion by 1 mL (10 units) is combined aseptically with 1,000 mL of a diluent	To control postpartum bleeding: 10 - 40 IU of OXT may be mixed to 1L of a diluent and run at a rate needed to control uterine atony. i.m. Administration of 1 mL (10 units) of OXT can be administered post-delivery of the placenta	i.v. infusion with physiologic saline solution, 500 mL, or 5% dextrose in physiologic saline solution
	Pfizer Canada Inc.	NS 5% Dextrose	10 IU of OXT are dissolved in 1000 mL of 5% dextrose solution	i.v. infusion. Administer 5 to 10 USP units via slow IV injection. Additionally, administer 5 to 10 USP units via IM injection.	NA

2.3.1.4 Cardiovascular protective properties

In animal research, OXT has become a quite effective cardioprotective agent. Often accompanying cardiovascular illnesses is disturbance of OXT and/or OXT receptor signalling. Commonly causing cardiovascular diseases, endoplasmic reticulum stress drastically lowered the OXT levels. By stopping the growth of atherosclerosis and healing the damaged heart, OXT can show cardiovascular preventive effect [25]. Moreover, cardiovascular problems help to explain the amazing morbidity and death among COVID-19 patients [26–28]. Notably, apart from the prevention of metabolic ailments accompanying diabetes mellitus and atherosclerosis. Reducing brain-heart syndrome and hypertension encouraging the regeneration of damaged cardiomyocytes helps OXT safeguard the heart and vasculature. Targeting different essential pathogenetic events in COVID-19, reports showed the cardiovascular protective properties of OXT [27]. OXT overcomes inflammatory cytokine release and neutrophil infiltration, stimulates T-lymphocytes, and counteracts the detrimental effects of angiotensin II as well as several significant pathogenic processes associated with COVID-19. Furthermore, OXT can reduce heparin and heparan sulphate fragmentation by increasing superoxide dismutase expression and stimulating γ-interferon expression to inhibit cathepsin L. Through these pathways, OXT can prevent viral invasion, inhibit the progression of multiple organ failure and ARDS (acute respiratory distress syndrome), decrease cytokine storm, and reverse lymphocytopenia. Exogenous OXT, in contrast to other treatment drugs, can be administered safely and does not cause the side effects observed in corticosteroid therapy or remdesivir.

Most importantly, OXT can be mobilised endogenously to stop COVID-19 from pathogenicity [29]. Among the several prospective therapies, OXT a typical hypothalamic neuropeptide with pleiotropic effects has become a powerful contender in the fight against COVID-19 [30].

2.3.1.5 Oxytocin in Brain Disorders

One alternative designation for the oxytocin receptor (OXTR) is a G protein-coupled receptor (GPCR), which plays an essential role in the intricate process of cellular signaling. It achieves this by binding to guanosine triphosphate (GTP), a key molecular player, and subsequently triggering a cascade of enzyme activities involving phospholipase C. The OXTR can be found throughout various regions of the human body, highlighting its significant presence and

functionality. In the brain, this receptor is particularly concentrated in several critical areas, each with specialized roles: the hippocampus, which is vital for the processes of memory formation; the head of the caudate-putamen, which governs the complexities of movement control; the ventromedial nucleus of the hypothalamus, important for regulating appetite and managing stress responses; and the central nucleus of the amygdala, which is a pivotal player in emotional processing [31].

As a neuromodulator, OXT exerts a profound influence over a wide array of central nervous system processes in both males and females, intricately shaping behaviors and physiological responses. The investigation into the role of OXT within the central nervous system has emerged as a fascinating area of research, capturing the attention of scientists. A growing body of preclinical and clinical studies highlights that the mechanisms of neurotransmission may have vital connections to the exploration and management of various neurological disorders, suggesting a promising avenue for future therapeutic interventions. OXT has shown benefit in some neuropsychiatric and degenerative conditions. These include social [32], non-social [33], emotional [34], sexual activity, behaviour, mating [35], sexual [31], epilepsy [36], affiliative [37], schizophrenia [38], obsessive-compulsive disorder (OCD) [39], mood disorders [40], Parkinson's disease, Alzheimer's disease (AD) [41], hypoxia, neuropathy, and others [42].

At the moment, 5 to 10 units/mL of OXT are offered in different regulatory markets for use by gynaecologists and obstetricians for treatment through intravenous (i.v) or intramuscular (i.m) injection. Because the molecule is hydrophilic and has a short half-life, only a small amount of OXT gets to the brain after being injected. Several clinical and animal studies have shown that giving OXT through the nose can help with a number of neurological diseases [43]. The reason for intranasal (i.n.) treatment is that it breaks down BBB. To fix the issues with BBB pervasion, different techniques have been tested to boost pharmacological reaction [44]. Strategies involving customised nano drug deliveries are important for getting OXT into the brain because they can lower its toxicity and boost its therapeutic effectiveness there [45]. In the next part, we'll talk about how OXT can be used to treat neurological disorders using both old and new carrier systems, and we'll also talk about what the future holds.

2.3.1.6 Neuropsychiatric disorders

Generalized social anxiety disorder & post-traumatic stress disorder

People have seen that OXT can help with anxiety, especially when it comes to social anxiety and fear. So, anxiety disorder is now another medical condition being looked into in OXT study [46, 47]. According to Hoge et al., plasma levels of OXT were seen to vary between individuals with healthy control (HC) and Generalised Social Anxiety Disorder (GSAD) [48].

The GSAD sample revealed a notable and beneficial connection between the heightened intensity of social anxiety symptoms, the participants' age, their gender, and the corresponding elevated stages of oxytocin (OXT). The authors of this report identified that individuals who experience significantly lower social satisfaction levels are more likely to have increased OXT levels circulating in their systems. The previously mentioned studies were specifically designed to assess and measure the peripheral levels of oxytocin in the participants [48].

Some studies looked at how i.n. OXT could help treat soldiers with post-traumatic stress disorder (PTSD) [48,49]. OXT was able to lessen the intensity of thinking that happened a lot during traumatic events. The patient's mood got better, and their worry level went down [48] [50]. Patients who got OXT treatment said they felt better about how they looked and how well they did in tests, especially when it came to public speaking. According to studies, giving OXT to people with social anxiety disease has been shown to help lower their levels of social stress. This decrease in social stress might have a bigger effect than just making anxiety feelings better. Because of this, people with anxiety problems may be more likely to get more out of psychotherapeutic exposure treatments [51,52].

Antidepressant effect

Many preclinical studies have been done since the first report of OXT's depressive effects and have found similar results. There is some evidence that OXT might help with depression, but this is only a guess because it controls neural activity by changing neurotransmitter release, reducing inflammation, and down-regulating the hypothalamic-pituitary-adrenal axis [53]. Central OXT can turn on GABAA receptors in the paraventricular nucleus and stop the stress that is caused by mRNA expression and corticotrophin-releasing hormone [54]. OXT also reduces depression and the negative consequences of excessively high glucocorticoid levels

[54]. OXT lowers immobility time in both chronic and acute clinical settings for the forced swim test [55]. Blocking antagonists of OXTR linked OXT antidepressant effect to this activity not evident in OXT receptor mutant animals [56–58]. The antidepressant activity of OXT was also validated in the tail suspension model. Consequently, the aforementioned trials validated the significant antidepressant impact of OXT [59].

Autism spectral disorder

OXT has demonstrated a beneficial influence on behaviors characterized by repetition, improvements in social connections, and the ability to recognize social cues in individuals diagnosed with autism spectrum disorder (ASD) [60]. A range of animal models has been carefully developed, and extensive preclinical studies have been conducted to thoroughly investigate the effects of OXT on human subjects with ASD [61,62]. Additionally, extensive research has been conducted using animal subjects to investigate the potential implications of OXT in understanding the underlying biological processes of ASD, revealing a substantial correlation with promising treatment targets for addressing this complex condition [63]. This notion is further reinforced by the widely acknowledged presence of social deficits, which are regarded as a fundamental aspect of ASD [64].

Initial study indicates that individuals with autism exhibit reduced plasma amounts of OXT. Numerous genetic studies indicate the significant significance of OXT in ASD. The OXTR genetic component is a significant candidate for future research due to its potential associations with ASDs. Within the framework of OXT, the CD38 gene emerges as a significant candidate gene for ASD. CD38's role in the production of OXT within the CNS is well established, and it has been noted to affect social conduct in mice [65,66]. The aforementioned reports regarding OXT and its impact on ASD are based on intravenous delivery [67].

Major depressive disorder & mood disorders

Clinical investigations have revealed a significant relationship between plasma OXT levels and the manifestation of depressive indications. In individuals diagnosed with Major Depressive Disorder (MDD), OXT concentrations were found to be markedly diminished when compared to those in control groups without depression [68,69]. This observation was further validated by multiple analogous studies focused on MDD patients, reinforcing the

findings [70,71]. Initial explorations into this association primarily aimed at evaluating OXT levels in individuals grappling with mood disorders, with a particular focus on depression [72]. Findings from these studies indicate that female patients tend to exhibit reduced OXT levels, whereas male patients may exhibit elevated concentrations. The connection between OXT levels and the experience of depression is intricate, heavily influenced by the interplay with other neurotransmitter systems, notably serotonin and dopamine (DA) [53,73]. There exists a noteworthy correlation between plasma levels of OXT and the diagnosis of MDD [40]. Following these initial findings, researchers sought to investigate how this correlation might relate to anxiety and other mood disorders, uncovering a similar impact of oxytocin in these contexts as well [74].

Couple bonding and sexual behaviour

Numerous studies have used rats as a preclinical model to explore the neurological connection of pair affinity. In these studies, OXT demonstrated enhanced community functioning in positive behaviours and improved, enduring connections following coupling [75]. The limbic system's robust links to the prefrontal cortex, which regulates intricate cognitive functions, significantly contribute to social attachment behaviours, such as couple bonding [76]. In individuals diagnosed with MDD who displayed sexual responses during night-time hours, their plasma OXT levels were significantly lesser than those observed in the control group. Even though there may be a surge of excitement during intimate and mating encounters, both anecdotal experiences and technical studies reveal that persons often feel a profound sense of drowsiness and tranquillity in the hours that follow sexual activity [75, 76].

Another experiment revealed that the release of OXT in the paraventricular nucleus of the central nervous system facilitated anxiolysis in male rats during sexual motivation and mating. One study indicated that OXT enhanced sexual and mating activity behaviour in male mice due to its antidepressant effects [56]. When a male and female were placed together in a cage with a perforated acrylic barrier, no direct physical interaction was seen; however, visual, auditory, olfactory communication occurred. The aforementioned investigations clearly indicate that OXT significantly influences sexual behaviour and tranquilly [57].

Schizophrenia & Obsessive-compulsive disorder (OCD)

Numerous researches have established that OXT significantly influences the pathophysiology of schizophrenia, particularly regarding memory function and social behaviours. Nonetheless, the precise mechanism behind its anti-schizophrenia action remains inadequately elucidated [77–79]. Alongside the goal of improving schizophrenia treatment through the investigation of novel molecular pathways, there is considerable interest in understanding the biological and clinical ramifications of OXT [38].

From a biological perspective, considering the dominant role of DA justification of psychosis, the obvious interaction between the DA and OXT systems suggests that OXT may have a role in this illness. OXT neurons have dopamine receptors in a variety of brain areas [79]. Additionally, it is noteworthy that OXT and DA receptors co-localize in mesolimbic system regions in relation to psychotic symptoms [80]. While early research suggested that people with schizophrenia had greater plasma levels of OXT, subsequent investigations were unable to replicate these results. Actually, recent research found no differences in OXT concentrations in CSF [80,81].

OCD is a persistent neuropsychiatric condition associated with varying degrees of depression [39]. Furthermore, OCD encompasses other subcategories of symptoms, including diminished insight, coexisting tics, and autistic behaviours [82,83]. It was brought to light in a case study involving hospitalized patients with severe OCD who, following four weeks of daily i.n OXT therapy, witnessed a marked improvement in OCD symptoms [84]. Additionally, the function of OXT in OCD patients was documented, and elevated OXT plasma levels in CSF were observed. These results suggest that OXT has a prospective function in OCD neutralization [82,83].

Epilepsy

Recently, OXT has been utilized in the brain through delivery methods based on nanoparticles (NPs), demonstrating encouraging outcomes in both *in vitro* (lab) tests and *in vivo* (animal) investigations [85]. The multidisciplinary field of nanomedicine holds great potential for assisting in this endeavour. When administered via i.n., OXT encapsulated in NPs proved more effective in treating epileptic animals than OXT administered alone in mice [86]. Exogenous OXT injection via the intranetural route has demonstrated some beneficial specific effects on

seizures [87,88], indicating that OXT's therapeutic benefits in the CNS of the brain may be enhanced by nano-based delivery methods.

In one study, the long-term use of OXT and OXT-loaded NPs prevented pentylenetetrazole-induced seizures. Apart from their anti-seizure properties, OXT-NPs also lessen hippocampus damage, apoptosis, and memory alteration. These results have demonstrated more effective ways to boost the therapeutic efficacy of OXT in animal models of epilepsy by employing nanocarriers via i.n. delivery [85]. On the other hand, there is currently a dearth of information about the therapeutic benefits of OXT-loaded NPs in epilepsy.

2.3.1.7 Neurodegenerative disorders

Alzheimer's disease

In meticulous evaluations conducted both in laboratory settings and within living organisms, OXT has revealed its remarkable ability to modulate the activation of microglia triggered by lipopolysaccharide, serving as a protective factor during the early phases of Alzheimer's disease [84,86]. Research has pinpointed a potential role for OXT as an anti-inflammatory agent within the brain, particularly relevant in the later stages of the disease, where inflammation often exacerbates cognitive decline. Pre-clinical investigations involving a variety of animal models have demonstrated that OXT not only enhances memory functions but also supports the assumption that it may show a vital role in the pathology of Alzheimer's disease. In studies involving two distinct cohorts of Alzheimer's patients, it was observed that the number of hypothalamic cells expressing OXT remained within normal ranges; however, intriguingly, there was a notable increase in OXT concentrations within the hippocampus and temporal cortex of these individuals [85,86]. Furthermore, elevated levels of oxytocin found in cerebrospinal fluid have been linked to a more favorable prognosis for those affected by Alzheimer's disease, suggesting a potential biomarker for monitoring disease progression [86].

Parkinson's disease

A deficiency in social interaction, coupled with an imbalance in the biological activities of OXT and/or DA, has been related to a variability of serious health conditions, comprising the eating disorder anorexia and the neurodegenerative disorder Parkinson's disease (PD). In individuals diagnosed with neurological conditions such as PD, the pathways governing central

dopamine and potentially OXT neurotransmission are profoundly compromised [89]. A growing body of research suggests that the cytoprotective effects of OXT could be mediated through multiple mechanisms, which include anti-inflammatory responses, prevention of programmed cell death (apoptosis), and protection against oxidative stress. In recent years, the rotenone-induced model of Parkinson's disease in rats has gained popularity among researchers as a valuable tool for exploring the intricate degenerative mechanisms associated with this disorder. Remarkably, OXT has been found to exert a robust cytoprotective influence, significantly reducing the occurrence of cell death in dopaminergic neurons that has been triggered by rotenone toxicity [87].

In a particular study that employed immunohistochemical assessment techniques, it was observed that rats with PD, which received saline treatments, displayed a notable reduction in the immunoreactivity of tyrosine hydroxylase a key enzyme in dopamine synthesis [90]. In stark contrast, a subsequent investigation revealed that the administration of OXT through the intranasal route caused in an upsurge in the appearance of tyrosine hydroxylase within the striatal neurons. Considering the critical involvement of caspase events in the mechanism of rotenone-induced cell mortality, the results from this research suggest that OXT plays a pivotal role in inhibiting apoptosis by modulating the caspase pathways and the death signals originating from the mitochondria. Comprehensive studies conducted in vitro and in vivo have underscored the significance of OXT in initiating neurogenesis and promoting cellular proliferation, as well as highlighting its pharmacological effects in alleviating the symptoms associated with PD [88].

Neuropathy

Researchers conducted a thorough investigation into the therapeutic potential of OXT when used in combination with liraglutide, a long-acting analogue of the human glucagon-like peptide-1 (GLP-1), utilizing an animal model specifically designed to study vincristine-induced neuropathy. Vincristine (VCR), a member of the vinca alkaloid family, is notorious for inducing a wide array of neurological deficits and is considered the most neurotoxic drug within its category due to its damaging effects on the nervous system. In their study, rats that were administered VCR underwent detailed histological and biochemical evaluations, revealing that both liraglutide and OXT significantly mitigated neuronal injury. This protective

response was attributed to a reduction in lipid peroxidation, a harmful process that damages cell membranes, alongside an enhancement in the expression levels of nerve growth factor, which plays a crucial role in the survival and growth of neurons [42]. The objective of the investigation directed by Erbas and co-workers was to assess the neurorestorative and neuroprotective effects of OXT administered i.p. on diabetic neuropathy in mice [71]. A comprehensive array of parameters, which included detailed electrophysiological evaluations, precise biochemical analyses, thorough histological examinations, and advanced immunohistochemical techniques, were meticulously analyzed to conduct this study. The results underscored the remarkable antioxidative and anti-inflammatory effects exhibited by various doses of OXT in the context of sepsis-induced neuropathy within rodent models. Findings from electromyography indicated that treatment with OXT could effectively rescue or restore neuromuscular function, with the outcome being contingent on the specific dosage applied. Following the administration of OXT, there was a significant increase in glutathione levels, while malondialdehyde levels demonstrated a notable decline, reflecting the treatment's efficacy.

The outcomes of the experiments reveal that OXT may effectively mitigate the detrimental effects caused by high blood sugar levels on peripheral neuronal cells. This protective action occurs through the inhibition of oxidative stress, inflammation, and programmed cell death pathways, particularly when OXT is delivered via intraperitoneal injection [91–93].

Hypoxia

Hypoxia refers to a condition where there is an insufficient supply of oxygen at the cellular level, leading to impaired functioning of mitochondria and consequently triggering neurodegeneration. This damage disrupts normal brain activity, which is closely allied with cognitive decline [94]. In a noteworthy study by Panaitescu and co-workers, it was discovered that administering OXT to rats experiencing seizures induced by hypoxia caused in a significant lessening in the total number of seizures the animals experienced [87]. OXT exerts a profound influence on the brain's neurotransmitter system, particularly on gamma-aminobutyric acid (GABA), which is known for its role in inhibiting neuronal excitability. By regulating the activity of GABAergic neurons, OXT helps to navigate the delicate balance

between depolarization and hyperpolarization, which can facilitate the typical low-oxygen environment experienced during labor and delivery. Furthermore, OXT has been revealed to show a vital role in alleviating inflammation within the central nervous system.

In one investigative study, researchers found that OXT therapy, when administered during a critical phase of severe hypoxia, resulted in long-lasting rehabilitative effects on seizures triggered by pentylenetetrazol, a substance that turns as an antagonist of the GABA (A) receptor. The study also observed a significant reduction in the levels of tumor necrosis factor-alpha (TNF-α) in both groups of hypoxic rats that received OXT treatment. This decrease in inflammation, along with the reduction in hippocampal gliosis, coupled with an enhancement in GABAergic activity, may provide insights into the mechanisms underlying these therapeutic effects [95–97]. The evidence was further supported by the considerable decline in TNF-α levels observed in both hypoxic groups treated with OXT, highlighting the hormone's potential neuroprotective properties. In the context of lipopolysaccharide (LPS)-induced brain inflammation, characterized by neuronal loss and the activation of microglia, the findings indicated that OXT effectively inhibited the activation of microglia, suggesting its protective role against neuroinflammatory processes [89,98].

2.3.1.8 Clinical Studies in Neurological Disorders

Presently, OXT has been used in various human clinical trials both alone and in conjunction with other treatments to treat a range of neuroleptic conditions. Various neural behaviours have been examined in clinical investigations with varying OXT doses (Table 2.3). Remarkably, scientists have reported a number of beneficial effects related to concurrent usage of OXT in neurodegenerative illnesses. The findings provide a new line of investigation for examining dose-dependent brain activity in relation to neurological conditions. Table 2.3 provides a concise summary of clinical research that provide insights into the effective interaction between OXT and the human neurological system in the treatment of neuropsychiatric diseases. In the same way, numerous patent applications that show the scientific evidence have been filed so far on OXT in relation to neurological complaints. Table 2.4 summarises key patent accounts detailing the efficacy of OXT in psychological diseases.

2.3.2 Vasopressin

Vasopressin (VP), a nonapeptide is a single linear chain molecule of nine amino acids, produced from enormous precursor proteins (propeptides), which in turn were derived from "prepropeptides," and comes under the class of macromolecules [110]. VP got first recognition in individuals and demonstrated high levels of blood pressure when administered with pituitary extract intravenously in the 19th century. Today, VP has recognition in major physiological events from maintenance of arterial pressure to edema in brain to restoration of kidney functions and management of neurological disorders [111]. The central nervous system (CNS) is affected by neurohypophyseal hormones i.e., VP, plays prominent role in learning, nociception, memory, central cardiovascular system regulation, social recognition, and stress response [112]. It has been challenging to pinpoint the precise target receptor responsible for the complex VP activities in the CNS. Possible targets reported are OTX-receptor gene, and three G-protein-coupled receptors (GPCRs) based i.e., V1a, V1b, and V2. Gq heterotrimeric GTP binding protein is activated by V1a, V1b, and OT receptors, and V2 excites Gs protein [113]. The primary VP receptors in the brain may be of the V1 type. However, some investigations have shown that the CNS may have a V2-like receptor. mRNA transcript autoradiographic distribution for the V1a and binding site autoradiographic distribution for the V1 were largely associated with receptors presence in CNS [114]. VP1 manages two important functions: body fluid content and preserves blood pressure. Two kinds of cell-surface receptors are involved in mediating the biological effects of VP. Adenylyl cyclase is connected to the renal receptor, i.e., V2, which is important in water reabsorption. The liver and vascular smooth muscle express the vascular receptor V1a [115].

Table 2.3: OXT to manage neurological ailments: Clinical summary

S.No.	Disorder/condition	Subjects	Method of study	Main Findings	Ref
1	Schizophrenia	27 Schizophrenia patients and 21 healthy controls	OXT levels in CSF	The patient and control groups had similar CSF OXT levels. OXT was inversely linked with second-generation antipsychotic dose and unpleasant symptoms.	[90]
2	Autism Spectrum Disorders (ASD)	10 ASD and 14 Healthy controls	OXT level in plasma pre and post psychosocial stressor	Adults with ASD exhibited elevated plasma levels of basal OXT	[99]
3	Schizophrenia	The study included 29 subjects (22 with schizophrenia and 7 with schizoaffective disorder) and 31 age-matched healthy controls	A self-administered intranasal dose of 40 IU of OXT or saline was given, followed by a structured clinical interview	A single i.n. OXT dose significantly improved the patient's social cognitive scores. However, healthy people did not experience the effect.	[100]
4	Schizophrenia	15 patients who are receiving regular antipsychotic treatment	The Positive and Negative Symptom Scale (PANSS) was assessed after adjunct daily doses of OXT (up to a maximum of 40 IU)	After receiving OXT for three weeks, patients' PANSS ratings significantly improved as compared to those receiving a placebo.	[101]
5	Schizophrenia	21 patients who are undergoing regular antipsychotic therapy	An emotion recognition test was conducted after adjunct daily doses of OXT (24 IU)	It was found that patients who got OXT were more adept at identifying emotions on both morphed and unmorphed faces.	[102]
6	Schizophrenia	21 patients (5 with polydipsia)	Emotion identification was assessed after receiving 10 IU or 20 IU of OXT, or a placebo, on three separate occasions	Emotional deficiencies at the basal level were negatively affected in patients who received 10 IU adjunct OXT. Conversely, 20 IU OXT reduced impairments in polydipsic individuals but not in a group of polydipsic individuals.	[102]

S.No.	Disorder/condition	Subjects	Method of study	Main Findings	Ref
7	Depression	10 Unmedicated and 10 healthy controls	Reading the Mind in the Eyes Test (RMET)	Patients' neural activity during the RMET was enhanced by a 40 IU dosage of OXT.	[94]
8	Postnatal depression	25 mothers with depression	Self-assessment and 5-minute speech sample	Mothers reported improved relations with their newborns after receiving 24 IU i.n. OXT	[95]
9	Schizophrenia	20 male patients	Functional magnetic resonance imaging (FMRI)	Patients with schizophrenia experienced lower levels of neural activity following 40 IU OXT. When a person with schizophrenia makes emotionally valenced social decisions, OXT reduces their perception of risk and/or aversion.	[96]
10	Post-traumatic stress disorder (PTSD)	18 PTSD Subjects	PTSD Symptoms Scale	OXT reduced the frequency and severity of thoughts related to the stressful incident. Anxiety was lessened and mood was enhanced.	[50]
11	Autism Spectrum Disorders (ASD)	29 children with ASD, 32 healthy children	Frequency-tagging EEG	Children with ASD who received repeated intranasal doses of OXT (12 IU, twice a day for 4 weeks) showed a significant improvement in neural sensitivity.	[97]
12	Autism Spectrum Disorders (ASD)	40 male subjects with ASD	Self-assessment and informant-based questionnaires	The study revealed a decline in social avoidance. improvement of repeated behaviours and mood.	[103]
IU: International Units					

Table 2.4: Patent insights on use of OXT in neurological disorders

S.No.	Patent No.	Disorder/condition	Main Findings	References
1	US3274060A	Multiple sclerosis	12 of the 21 patients treated with this invention exhibited an instant improvement, increasing their scores from 3 to 50 points; three more patients showed an increase from 50 to 100 points; three more patients had an improvement above 100 points; and only 3 patients exhibited no change or regression. It should be noted that a score increase of 100 points is represented, for example, by a shift from -300 to -200	[104]
2	WO2004/078147A2	Neuroleptic disorders	Controlled release OXT formulation has been developed and evaluated for effectiveness, particularly in conditions involving neuroleptic disorders. It was stated that formulation would improve OXT transit across the blood-brain barrier and increase stability	[105]
3	WO2008042452A1	Autism spectrum disorders	The use of OXT and its analogs in the prevention and treatment of autism spectrum disorders has been demonstrated by invention	[106]
4	US11291626B2	Neurological conditions and disorders	The invention describes a drug delivery device for intranasal administration of OXT to the upper posterior nasal airway, aimed at treating neurological conditions.	[107]
5	US20160310683A1	Social cognitive behaviours	The current invention proposed that administering individuals 24 IU of OXT intravenously would enhance their social and cognitive behaviours	[108]
6	US20130085106A1	Psychiatric disorder	A method was developed to treat mental illnesses in humans by giving i.n. OXT at a dose which is effective. Furthermore, research suggested a method to prevent individuals receiving opioid medication for pain relief from developing opioid dependence, tolerance, or withdrawal symptoms	[109]

V1b, a third VP receptor, is thought to be expressed only in pituitary cells. These freshly discovered receptors all belong to the "seven membrane-spanning" receptors that communicate with G proteins [116]. V1b receptor is localised in *Cornu Ammonis* (CA2) pyramidal neurons in the olfactory bulb, suprachiasmatic, hippocampus, dorsomedial hypothalamic nuclei, supraoptic, entorhinal cortices, piriform, substantia nigra, dorsal motor nucleus of the vagus brain regions. It has been demonstrated that VP/OT peptides interaction with their receptors offer a variety of chances to modify the effectiveness of sensory transmission [117].

Within minutes of VP binding, cells experience homologous desensitization. From the cell surface, VP receptors are ingested and recycled. It is still debatable how receptor internalization affects VP signalling. Recent research has shown that the activation of some signalling pathways depends on the receptor internalization of other receptors that are signalling through G-proteins [118]. VP has been demonstrated to be a strong vasoconstrictor in VSMC as depicted in in vivo and cultured cell preparations. Following exposure to the hormone, increase in contractile force are seen within seconds to minutes and are mediated through the V1a receptor [119].

2.3.2.1 Chemistry, biogenesis and pharmacology

VP is produced as a precursor hormone of substantial size within the supraoptic nuclei and paraventricular located in hypothalamus. Approximately 10 to 20% of overall VP reservoir located in the posterior pituitary is promptly discharged initially, followed by a subsequent secretion in response to suitable stimuli. The complete sequence of VP synthesis, transportation, and storage within the posterior pituitary gland requires around 1 to 2 hours [120]. The primary factors that elicit the release of VP are heightened plasma osmolality and significant hypovolemia. The release of VP is also augmented by CNS input triggered by pain, nausea, hypoxia, pharyngeal stimulation, as well as endogenous and exogenous substances. The release of VP is strongly stimulated by hyperosmolality, which is detected by both central and peripheral osmoreceptors [121].

The exogenous VP, is available as a sterile solution intended for administration through i.m., i.v. and s.c. routes. VP does not exhibit protein binding and possesses a volume of distribution

measuring 140 mL/Kg and half-life of 24 mins [122]. It undergoes renal elimination, accounting for 65% of its clearance while the remaining 35% is attributed to its metabolism by tissue peptidases [123]. The presence of VP deficit may be a contributing factor to occurrence of hypotension in patients who exhibit cardiac arrest and shock due to post-cardiotomy and catecholamine resistance. The administration of VP infusions results in plasma concentrations ranging from 20 to 30 $pg.mL^{-1}$ that can elicit a pressor response while minimising the occurrence of organ hypoperfusion [124]. The i.v. administration of low dosages of VP effectively restores vasomotor tone while causing little ischemic effects on renal, mesenteric, pulmonary, and cardiac systems. Nevertheless, it is imperative to conduct clinical trials to ascertain the impact of VP in clinics to evaluate organ failure, long-term prognosis, and mortality rates [125].

The release of VP is effectively inhibited by afferent vagal impulses initiating from the stretch receptors in the left atrial, carotid sinus and aortic arch. Atrial receptors are activated in reply to elevations in blood volume. In contrast, the receptors located in the aortic arch and carotid sinuses are stimulated by rises in arterial blood pressure [126]. The release of VP is increased during severe hypotension as a result of decreased activity in arterial baroreceptors. The impact of central venous pressure levels doesn't lift VP release until there is a decrease in mean arterial pressure [127]. The production of VP is transiently inhibited by volume expansion and significant rises in blood pressure, which is mediated via the atrial stretch receptors. Various neurotransmitters and hormones, such as acetylcholine (acting through nicotinic receptors), dopamine, histamine, prostaglandins, other catecholamines, and angiotensin, can induce the production of VP directly [128]. Elevated levels of partial pressure of carbon dioxide ($PaCO_2$) serve as a stimulus for the activation of carotid body chemoreceptors, thereby leading to an elevation in VP levels [129]. The inhibition of VP is observed by the action of atrial natriuretic peptide, c-aminobutyric acid, and opioids via α1-adrenoreceptors [130]. On the other hand, the inhibition of VP and oxytocin production is mediated by α2-adrenoreceptors and β-adrenergic receptors. The typical fasting VP concentration in humans is less than 4 $pg.mL^{-1}$. Minor elevations in plasma osmolality are promptly detected, leading to a subsequent rise in

concentration of VP 10 pg. mL^{-1}. The levels of VP equal to or greater than 20 pg.mL^{-1} elicits the maximum rise in urine osmolality. The duration of VP's half-life ranges from 10 to 35 minutes. The compound undergoes fast metabolism by hepatic and renal passes [131]. The ultimate shared route of VP release is in the paraventricular nuclei, specifically in the cell bodies of magnocellular neurons. Subsequently, VP is transported to posterior pituitary glands pars nervosa via supraoptic-hypophyseal tract (Figure 2.2). Plasma hypertonicity leads to the depolarization of magnocellular neurons in the hypothalamus, resulting in an increased release of VP. Hypovolemia results in a significant rise in VP levels, which is facilitated by the presence of hypotension and reduced intravascular volume. VP release is elevated through a shift of the osmolality-VP response curve in an upward and leftward direction. In hypovolemic situations, it is necessary to have elevated amounts of plasma VP in order to uphold appropriate osmolality [132].

2.3.2.2 Pharmacokinetics of Vasopressin

Animal research has revealed that vasopressin undergoes metabolism through the action of specific enzymes, including serine proteases, carboxypeptidases, and disulfide oxidoreductases. This metabolic process primarily takes place within the liver and kidneys, two vital organs known for their role in processing substances in the body. These enzymes cleave the vasopressin molecule at strategic sites that are critical for its biological activity. As a result, the metabolites generated from this breakdown are anticipated to be inactive, meaning they do not exert the same physiological effects as the original hormone. Ultimately, the body eliminates vasopressin through the urine, with a mere 6% of the administered dose being excreted in its unchanged form, highlighting the efficiency of the metabolic process in breaking down this important hormone.

2.3.2.3 Role of V1 and V2 receptors

The potential pharmacological effects of VP receptors can be attributed to the distribution and density of VP receptors, which exist in three distinct subtypes [133]. V1 or V1a are vascular receptors with wide presence on vascular smooth muscle and initiates vasoconstriction. V1 receptors are present in various anatomical locations, including the kidney, bladder,

myometrium, spleen, hepatocytes, adipocytes, platelets. Vasoconstriction mediated by V1 receptors involves the activation of Gq proteins, which in turn activate phospholipase C (PLC). This enzyme releases calcium ions (Ca^{++}) from intracellular reserves through production of inositol triphosphate and diacylglycerol [130,134].

V2 renal receptors initiates antidiuretic effects due to VP presence [135,136]. V3 receptors show a vital part in mediating effects of VP presence on the CNS. These receptors function as neurotransmitters or modulators, regulating several physiological processes such as blood pressure, memory, body temperature, and pituitary hormone release [137]. V2 is responsible for maintaining homeostasis, participating in thermoregulation, facilitates memory, sleep processes, and release of ACTH [138]. In typical physiological circumstances, the primary physiological function of VP is management of homeostasis for water [139]. In the context of normal physiological conditions and at concentrations within expected range, VP does not exert a substantial influence on regulation of blood pressure in vasculature [140].

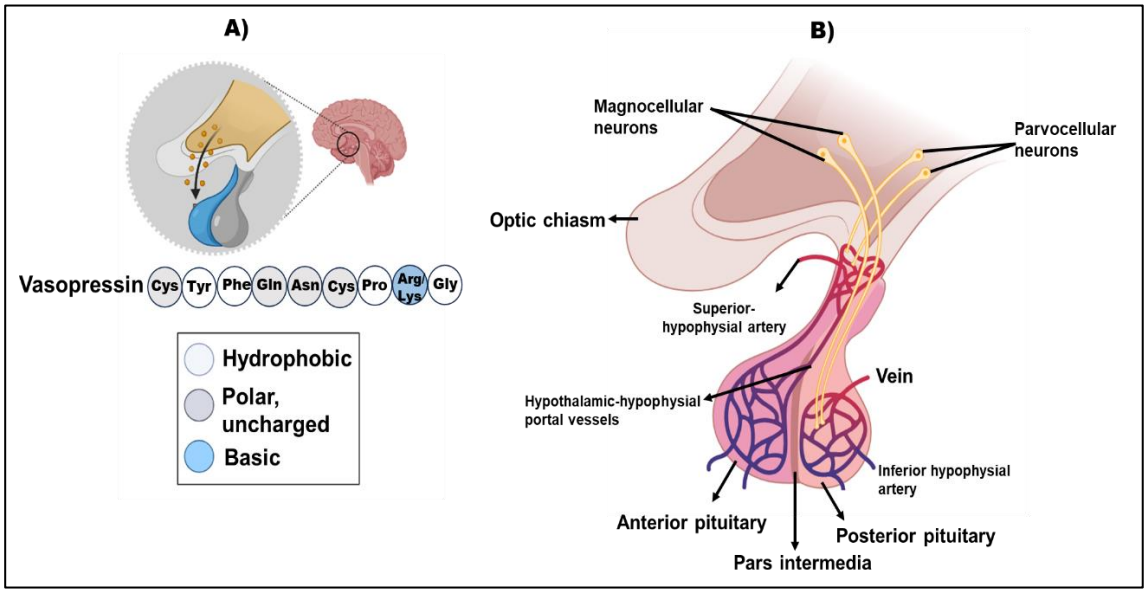

Figure 2.2: A) Chemistry of vasopressin and B) site of its release from the pituitary gland

VP exhibits little vasopressor activity in animals possessing an intact autonomic nervous system. The administration of substance induces a shift towards left in the baroreflex curve of

heart rate-arterial pressure, mostly through its interaction with V1 receptors located in brain [141,142]. VP additionally enhances vasculature's responsiveness to norepinephrine. The action of VP involves inhibition of potassium-sensitive adenosine triphosphate (K-ATP) channels, with degree of inhibition being depending on dosage administered [143]. Induction of vasodilation is due to inhibition of Ca^{++} channels and K-ATP channel activation. Furthermore, it has been observed that at physiological doses below 10 pg. mL^{-1}, this substance exhibits vasoconstrictive effects on mesenteric circulation. This phenomenon is believed to be mediated through V1 receptor [144]. In addition to its other effects, VP also augments urine concentration by many processes [145,146]. These include the activation of a specific urea transporter, which leads to an upsurge in the medullary concentration gradient. The administration of pharmacological amounts of VP in male's results in a rapid elevation of cortisol levels in the bloodstream [147].

The key factors influencing the effects of neuromodulators are modulator chemical properties, concentration, and receptor spatial arrangement in brain. By virtue of the ability of VP and other neuropeptides to access any target neuron, the capacity of neurons to process and handle information is significantly augmented. The heuristic importance of differentiating VP's roles as a neuromodulator, neurotransmitter, and hormone lies in its ability to elucidate the mechanisms behind spatial accuracy, and potentially limitless flexibility in signalling [148]. The receptors of brain V1 type exhibit positive coupling with PLC, hence exerting their effects through activation of phosphoinositol turnover and intracellular mobilisation of Ca^{++} [149]. On the other hand, V1b subtype was observed in the anterior pituitary and genesis of ACTH secretion. V1a is considered as the brain's primary VP receptor. The presence of V1b and V1a receptor transcripts was observed in the supraoptic nucleus (SON) of the hypothalamus, as well as in both parvocellular and magnocellular neurons (MCN) of the paraventricular nucleus (PVN) [40].

The V1 receptor in brain is unaffected by alterations in circulating ovarian hormones or plasma osmolality, especially in context of brain. Conversely, empirical data suggests that the upregulation of V1b receptor mRNA occurs subsequent to prolonged exposure to stressors

[150]. The involvement of endogenous VP in stress coping and stress-related diseases is contingent upon two factors. Firstly, it must be released within certain brain regions, particularly in response to exposure to stressors. Secondly, its effects mediated through receptors should be likely to contribute to effective stress-coping techniques. This neuropeptide satisfies both of the conditions [151]. Hypothalamic neurons release of VP occurs from both their dendrites and somata [152]. Various factors influence the specific source of VP outside hypothalamus. These factors include the density and activity of dendritic and axonal fibres [153]. VP levels in amygdala are regulated by the process of dendritic release and subsequent diffusion, potentially influenced by local axonal release. Although the synaptic release from axonal terminals may not be quantitatively equivalent to dendritic release under basal conditions, it plays a crucial role in neuromodulation [154]. This is due to its ability to provide a greater quantity of ligands for receptor binding and promote temporal and spatial signalling. For instance, when subjected to forced swimming, which serves as a stressor that encompasses both emotional and physical aspects, VP release occurs in the SON and PVN through dendritic mechanisms [155]. Notably, this neuropeptide's systemic secretion is unaffected by forced swimming. The experience of social defeat, which is a very distressing emotional stimulus, induces the secretion of VP specifically within PVN of the brain rather than in SON, septum, or posterior pituitary gland for systemic circulation [156, 157].

Thapsigargin and cyclopianzonic acid are compounds that induce calcium ions (Ca^{2+}) release from the intracellular stores of the endoplasmic reticulum. These compounds have been found to stimulate the release of VP from magnocellular neurons. Additionally, thapsigargin has been observed to significantly enhance the release of VP from dendrites, particularly when stimulated by osmotic or high potassium conditions. The observed effect exhibited a prolonged duration, was contingent upon the passage of time, and showed specificity towards cyclopianzonic acid and thapsigargin. These findings indicate a priming process of dendritic VP, which enables future release upon electrical and depolarization-induced activation. Surprisingly, the administration of thapsigargin did not result in enhancing secretion from the

axon terminals. While VP demonstrates efficacy in triggering the release of dendritic VP, it does not effectively produce priming [158].

2.3.2.4 Cerebrospinal fluid and Vasopressin

VP has the potential to access the ventricular cerebrospinal fluid (CSF) through a minimum of three distinct pathways. There are three potential mechanisms by which VP may be distributed within the body [159]. Firstly, via bloodstream transportation; secondly, VP is directly released into CSF through VP-ergic endings; and thirdly, VP is released from ventricles via transependymal routes [160]. In research reports it was observed that administration of concentrated human CSF intravenously containing VP and OXT to rats resulted in release of both hormones in brain [161].

Jenkins and Mather *et al.* have revealed elevated concentrations of VP in CSF of the patient's diagnosed with subarachnoid haemorrhage [162]. These studies found that CSF VP levels were particularly elevated in patients who had altered levels of consciousness, which may indicate an underlying rise in intracranial pressure [163]. The potential involvement of CSF atrial ventricular protein modulates water content and intracranial pressure (ICP). CSF VP levels in individuals diagnosed with pseudotumor cerebri or benign intracranial hypertension was prompted due to the potential involvement of alterations in water content leading to high ICP in patients [164]. Sorensen and colleagues conducted a study in which they assessed CSF and plasma VP concentrations in a group of 10 patients who met the standard criteria for benign intracranial hypertension. These measurements were then compared to the values obtained from a control group of 28 patients who had cervical or lumbar disc syndromes. The average concentration of VP in CSF was found to be considerably greater in patients diagnosed with benign intracranial hypertension (2.2±0.3 pg/mL) compared to the control group (1.3±0.1 pg/mL). However, no significant difference in mean plasma VP concentrations was seen between the two groups. The study did not provide a definitive response regarding whether the elevated CSF VP observed in benign intracranial hypertension is a causal factor or a consequence of the heightened intracranial pressure [126]. Reid and Morton also observed increased CSF VP levels in individuals with benign intracranial hypertension [165].

Sorensen and colleagues conducted a study to examine levels of CSF VP in two distinct groups of patients affected by dementia. The first group consisted of 16 patients diagnosed with primary symptoms of degenerative dementia of Alzheimer's disease (AD) type. In comparison, the second group comprised of 18 patients with normal hydrocephalus and reversible dementia. The concentration of VP in CSF was lowered in patients diagnosed with degenerative dementia in comparison to patients with normal hydrocephalus and individuals without any neurological disorders [166,167]. Sundquist *et al.* studied patients with alcohol-induced dementia have been found to exhibit decreased levels of VP in their CSF [168]. Tsuji *et al.* discovered that individuals diagnosed with dementia had elevated levels of CSF VP compared to a control group. It is worth noting that all control participants had CSF VP concentrations that fell below the detection threshold. The observation of reduced CSF levels of VP in individuals with degenerative dementia may appear to support the hypothesis implicating VP in learning and memory functions [169].

Similarly, the identification of reduced CSF VP levels in Parkinson's disease also aligns with this line of evidence [170]. Necropsy examinations conducted on the brains of individuals who passed away with a confirmed diagnosis of AD revealed minimal or negligible variations in VP levels across several brain regions in comparison to the brains of control subjects. There were no remarkable variances observed in the stages of VP immunoreactivity in the locus coeruleus and substantia nigra between post-mortem samples from individuals with Parkinson's disease and control brains [171,172].

The studies revealed that CSF concentrations of VP in individuals with multiple sclerosis did not exhibit any significant variances when equated to the values observed in control groups. Pedersen et al. conducted a study to assess CSF VP levels in patients diagnosed with CNS metastases originating from bronchiolar carcinoma of small cells. The study revealed a notable rise in the ratio of CSF to plasma VP, as well as raised concentrations in individuals suffering from leptomeningeal carcinomatosis. Nevertheless, it should be noted that elevated plasma levels did not consistently correspond to elevated CSF levels [173]. Additionally, the ratio of VP in CSF to plasma in patients with a syndrome of unsuitable antidiuretic hormone secretion

(SIADH) did not differ from that observed in control individuals [174]. The observation above was earlier documented by Luerssen and Robertson, who additionally discovered a statistically significant elevation in the concentration of VP in CSF of individuals diagnosed with diabetes insipidus. Sorensen et al. conducted a study in which they observed a significant elevation in CSF VP levels in a patient ten days following hypophysectomy [129].

Gold *et al*. proposed a hypothesis signifying the potential participation of VP in the pathophysiology of effective ailments. Subsequently, they conducted a study and showed that individuals diagnosed with bipolar manic-depressive psychosis exhibited significantly lower levels of CSF VP during depressive episodes compared to manic episodes. CSF amyloid-beta (Aβ) precursor protein levels were found to be considerably lesser in the depressed group as a whole in comparison to the control group. Sorensen et al. provided empirical evidence that suggests alterations in the central release of VP may be associated with effective disorders. Authors also observed a decrease in the plasma VP response when individuals with depression were administered with hypertonic saline, as compared to those with mania. Within this particular framework, it is important to highlight the findings of Sorensen *et al*., who discovered that electroconvulsive therapy (ECT) elicited a significant, although temporary, release of VP. Furthermore, they observed that patients who exhibited a satisfactory response to therapy saw an elevation in their baseline plasma VP levels [175]. The observed correlation between impairments in several peptides and hormone activities in individuals with depressive illness may perhaps be a coincidental parallel event. Sorensen and colleagues conducted a study examining CSF VP concentrations in individuals diagnosed with schizophrenia, including those with acute or chronic illness [129]. Higher levels of CSF VP have been seen in various cohorts of individuals with brain diseases characterised by heightened intracranial pressure. Furthermore, the concentration of VP in CSF exhibits a direct correlation with the magnitude of ICP. Nevertheless, it is imperative to evaluate the potential impact of elevated amounts of CSF VP on intracranial pressure. Degenerative disorders have been associated with diminished levels of CSF VP, likely due to widespread neuronal loss, including the VPergic systems [168].

2.3.2.5 Vasopressin in neurological disorders: Clinical and preclinical studies of Vasopressin

Alzheimer's disease (AD)

Vasopressin (VP), a crucial neuropeptide, is synthesized in the hypothalamus, a part of the brain that plays a vital role in various regulatory functions. The distribution of VP receptors is noteworthy, as they are predominantly located in specific brain regions, including the amygdala, which is integral to emotional processing; the hippocampus, essential for memory formation; and the bed nucleus of the stria terminalis, allied with stress responses. Additionally, VP receptors can also be found in selective areas of the prefrontal cortex, tangled in decision-making and advanced cognitive functions, as well as the cingulate cortex, which is linked to emotional regulation and pain.

Extensive research has revealed that VP actively blocks the process of memory extinction, which is the gradual loss of a conditioned response, and it has the capability to reverse amnestic effects caused by various experimental interventions. Notably, the administration of peripheral doses of VP has been shown to enhance memory acquisition, illuminating a potential therapeutic avenue for memory-related disorders. However, this increase in memory performance often comes with a notable side effect: systemic hypertension, or elevated blood pressure, which raises concerns about the safety of such treatments. The mechanism behind VP's positive influence on memory may stem from its ability to indirectly heighten levels of arousal, creating a more conducive environment for memory formation and retrieval. Intriguingly, the antihypertensive effects of VP its capacity to lower blood pressure are obstructed by centrally acting anticholinergic medications, which target the central nervous system. In contrast, peripherally acting anticholinergic drugs do not exhibit the same blocking effect. This distinction indicates that VP’s enhancement of memory might primarily involve central nervous mechanisms, underlining the complex interplay between hormonal activity, memory processes, and cardiovascular responses within the brain.

VP has been identified as a regulator of male-typical social behaviours, scent marking, encompassing paternal care, pair bond formation, selective aggressiveness, and social

recognition memory. Elevated concentrations of VP in the CSF have been linked to heightened aggression among individuals with psychiatric disorders [176]. VP may have the ability to influence the neural networks involved in the regulation of emotions, particularly fear [177]. Post mortem study of 12 patients diagnosed with AD and 13 normal patients, showed lower levels of VP in AD brain tissues, especially regions of hippocampus, globus pallidus and nucleus accumbens [177]. In a study by Zhang and coworkers, i.n. VP administration with a dose of 2 µg/Kg (tid) for 4 weeks in amyloid precursor protein (APP)/PS1 mice AD model revealed enhancement in spatial and working memory in the mice [179].

In brief, the findings from preclinical investigations indicate that VP has the potential to facilitate the process of memory acquisition and retrieval. The role of Vasopressin in various neurological disorders is compiled in Table 2.5 with preclinical or clinical inferences.

Obsessive-compulsive disorders (OCD)

Significant anatomical interactions between VP and serotonin (5-HT) have been documented. The neurotransmitter 5-HT is likely to induce the secretion of VP through activating 5-HT2A and 5-HT2C receptors. The administration of VP congeners has been found to be correlated with the improvement of memory in both individuals who are in good health and those who have minor cognitive impairment. Although the observation of this phenomena has not been consistent across all studies, variations in the results may be attributed to the specific type of VP used, as well as variations in dosage and method of administration. Des-amino-8-D-arginine VP (DDVP), a synthetic analogue of VP, has demonstrated the ability to modify memory retrieval in children diagnosed with attention and learning impairments. However, a significant difference in cognition was not observed when i.n. administration was studied clinically [180]. The administration of VP through the intranasal route results in a significant elevation of mismatch negativity observed in event-related potentials among individuals without any medical conditions. This increase signifies an enhancement in the automatic processing of stimulus deviance [185].

Table 2.5: Clinical and preclinical studies of Vasopressin

S.No.	Title of study	Study (Preclinical / Clinical)	Dose/Route of administration	Inference	Reference
1.	Double-blind randomized trial on 41 males	Clinical	20 IU/ i.n.	Acceleration of the processing of sexual cues and an elevation in salivary cortisol levels during the Trier Social Stress test	[176]
2.	40 Males and Females	Clinical	40 IU/ Nasally	Influence the neural networks involved in the regulation of emotions, particularly fear	[177]
3.	Postmortem of patient brain tissue	Clinical	-	Patients suffering from AD reported lower brain levels of VP	[178]
4.	APP/PS2 mouse AD model	Preclinical	2 µg/Kg (tid) for 4 weeks/ i.n.	Enhancement in spatial and working memory	[179]
5.	Rat grooming behaviour	Preclinical	-	Paraventricular hypothalamic vasopressin activation was observed with self-grooming behavior	[180]
6.	Comparison of plasma VP in OCD and healthy individuals	Clinical	-	Levels of VP in basal plasma was higher in OCD patients administered with hypersaline solution	[181]
7.	Study of molecule SSR149415 in murine model	Preclinical	-	Elevated levels of V1a-binding sites in the lateral septum	[182]
8.	Plasma VP levels in social active and depressed patients	Clinical	-	VP potentially beneficial impact on mitigating the cognitive impairments frequently observed in individuals with depression	[183]

S.No.	Title of study	Study (Preclinical / Clinical)	Dose/Route of administration	Inference	Reference
9.	VPr1b gene expression in depressed patients	Clinical	-	SNPs in the VPr1b gene among individuals diagnosed with mood disorders and mutation in amino acid	[183]
10.	Investigation of SNPs in the VPr1b gene in Belgium and Swedish depressed patients	Clinical	-	Significant correlation was observed in Swedish (SNP VPR1b-s3) and Belgian (SNP VPR1b-s5) with recurrent patients	[184]
11.	9 patients administered with DDVP diagnosed with Downs syndrome	Clinical	40 µg/day for 10 days/ i.n.	Administration of DDVP did not result in an improvement in Word List Memory	[185]
12.	VP receptors in individuals with ASD within the Korean population	Clinical	-	Studies revealed a noteworthy correlation between ASD and SNPs RS1 and RS3, which are located in the 50 flanking areas of the V1aR receptor	[183]
13.	VP administration in men and children with ASD	Clinical	4 week/ i.n.	VP has potential in amalgamation of ASD	[61]
14.	Social behaviour in V1aR knockout mice and VP-deficient rats	Preclinical	-	Post-mortem investigations have indicated a decrease in VP levels inside the temporal brain of individuals diagnosed with schizophrenia	[186]
15.	DDVP in 40 patients with schizophrenia	Clinical	20 µg/day for 8 weeks/ i.n.	Effective treatment was observed and neutralization of chronic schizophrenia	[187]

The injection of VP has been found to increase self-grooming behaviour. The studies were done preclinically in which hypothalamic vasopressin was administered to mice, and it was observed that self-grooming behaviour was increased manifold times and significantly [188]. This finding serves as an additional piece of evidence that suggests a possible involvement of the neuropeptide in the regulation of certain symptoms linked with OCD. The recurrent act of handwashing and the strict adherence to personal hygiene rituals, which are frequently observed in individuals with OCD, can be analogized to the self-grooming behaviour exhibited by animals. The self-grooming behaviour in rats is observed to enhance following the administration of VP through both intraventricular and intraamygdala infusions. As per clinical investigations, it has been shown that individuals diagnosed with OCD may have irregularities in VP functioning. In their study, Altemus *et al.* (1992) conducted a comparison between adult patients diagnosed with OCD and a group of healthy individuals. The researchers studied the levels of VP in the basal plasma and CSF, as well as the alteration in plasma VP resulting from the administration of hypertonic saline. It was observed that patients exhibited a considerably higher secretion of VP into the plasma following the administration of hypertonic saline compared to the control group [188].

Additionally, another research unveiled a noteworthy positive association between CSF concentrations of VP and homovanillic acid (HVA), which serves as the principal metabolite of dopamine (DA) in the central nervous system [189]. In contrast, the study conducted by Leckman and coworkers found no statistically significant variation in CSF levels of VP among a sample of 39 people diagnosed with OCD, 33 patients diagnosed with Tourette syndrome (TS) (including 14 individuals with comorbid OCD and TS), and 44 individuals without any psychiatric disorders serving as the control group. Nevertheless, a notable increase in CSF concentrations of oxytocin was observed in individuals with OCD who did not have a personal or familial background of disorders in comparison to the remaining cohorts [190]. Swedo *et al*. (1992) conducted a study on adolescents and children diagnosed with OCD and found a statistically significant reverse relationship between the intensity of obsessive-compulsive symptoms and the levels of VP in the CSF. There was a adverse connection observed between

the ratio of CSF, VP, to OXT and the severity of OC symptoms. When taken into account alongside the discovery of increased CSF levels of VP in certain adults diagnosed with OCD. The findings indicate that significant developmental alterations may take place in CSF markers of VP in individuals with OCD. Although the precise impact of VP on OC symptoms has yet to be determined [191].

Depression and anxiety

Anxiety frequently manifests as a fundamental symptom in individuals diagnosed with depression, and there exists a notable prevalence of co-occurrence between anxiety and depressive disorders. Furthermore, it has been observed that anxiety disorders often occur before the onset of depression, indicating a potential connection between both diseases and shared underlying physiological characteristics. Since both diseases involve maladaptive responses to stimuli, they are considered stress-related disorders, indicating a potential causal relationship with dysregulation of the hypothalamic-pituitary-adrenal (HPA) system. Animal models are frequently employed in research to understand the underlying causes of depression better [49].

Consequently, these models often exhibit changes in behaviour that are associated with anxiety. With CRF neural circuits, it is plausible that CRF may primarily be associated with the manifestation of anxiety symptoms observed in a significant number of individuals with depression. The observed heterogeneity in depressed patients' HPA system function may be attributed to the varying presence of anxiety symptoms among individuals [192]. In addition to the excessive activity observed in central corticotropin-releasing hormone (CRH) neuropeptidergic circuits, it is supposed that VP neuronal systems add causally to the development and manifestation of anxiety disorders. The administration of VP has been observed to produce various behavioural effects, including heightened anxiety levels after being administered intracerebroventricularly [193].

Additionally, VP has been found to enhance the release of ACTH from pituitary corticotrope cells when triggered by CRH. Following extended periods of stress, VP has an augmented expression and release from hypothalamus neurons in both human and rodent subjects. In a

study successfully demonstrated the role of hypothalamic VP in the dampening of the HPA system mediated by benzodiazepines [181]. The results of clinical trials indicated a substantial correlation between plasma VP concentrations and anxiety-related symptoms in healthy volunteers who were subjected to an anxiogenic drug challenge. Additionally, increased levels of plasma VP were observed in individuals diagnosed with depression. Consequently, the utilization of the non-peptide VP 1b (V1b) receptor antagonist SSR149415 showed anxiolytic and antidepressant-like properties in animals [182]. The VP 1a (V1a) receptor, recognized for its significant expression in the lateral septum, thalamic nuclei, and the amygdalo striatal transition area in rats, has been broadly considered for its involvement in several behavioural processes, including the regulation of emotionality and stress coping mechanisms. The experimental findings by Gal indicate that the administration of septal VP has been demonstrated to elicit an elevation in anxiety-related behaviours among rats. Consequently, rats who exhibited an intrinsic predisposition towards higher levels of anxiety demonstrated elevated levels of V1a-binding sites in the lateral septum in comparison to rats with lower levels of anxiety. Elevated levels of intra-PVN VP were observed in the rats exhibiting high levels of anxiety. Furthermore, the injection of paroxetine, an antidepressant widely used in clinical practice, effectively restored abnormal behavioural and neuroendocrine patterns in this animal model of psychopathology. This study provides evidence that an upregulation of hypothalamic VPergic activity, resulting from a compromised repression at an VP promoter polymorphism, contributes to the dysregulation of the hypothalamic-pituitary-adrenal (HPA) system. Moreover, the observed decrease in VPergic overexpression following treatment with paroxetine suggests that this neuropeptidergic system plays a vital part in the mechanism of action of antidepressant medications known for their efficacy in treating anxiety disorders [182].

Patients diagnosed with either generalized anxiety disorder (GAD) or severe depressive disorder were subjected to the administration of the VPr1b antagonist, SSR149415. The results of this clinical research indicate that the administration of SSR149415 did not yield significant improvements in persons diagnosed with generalized anxiety disorder (GAD) [182]. However,

it was observed that after 8 weeks, the medication did exhibit antidepressant benefits in patients diagnosed with major depressive disorder. Although numerous research studies have documented increased levels of VP plasma concentrations in individuals with depression, there exists a lack of consensus within the scientific community regarding this phenomenon. In a study directed by Sorensen and Gjerris *et al*., it was witnessed that there were no discernible variations in the plasma concentrations of VP between those diagnosed with depression and those who were deemed healthy controls [153].

The subsequent investigations have endeavoured to ascertain the particular characteristics linked to depression that exhibit a correlation with concentrations of VP. An instance of heightened plasma VP has been identified in individuals experiencing depression accompanied by psychomotor slowness during daytime and increased motor activity during night time [194]. Moreover, a subset of individuals experiencing depression exhibits a significant association between memory function and concentrations of VP. This finding implies that VP may have a potentially beneficial impact on mitigating the cognitive impairments frequently observed in individuals with depression [194]. The studies indicate that the basic concept proposed by Gold regarding the decrease of VP levels in depression and increase in mania may not be entirely accurate. Although there is evidence suggesting that various subgroups of individuals with depression exhibit abnormalities in the VPergic system [175].

Genetic association studies have been employed to establish a potential correlation between alterations in the VP system and depression. Dempster and co-workers assessed the impact of single nucleotide polymorphisms (SNPs) in the VPr1b gene among individuals diagnosed with mood disorders that originated during childhood. The discoveries of this study demonstrate alterations in the amino acid Lys65Asn. The authors hypothesized that this modification influences the activation of PLC, leading to a disruption in the ability of corticotropin to modify VP binding [195]. Van West *et al*. conducted a study on depressed individuals from two distinct populations (Belgian and Swedish) to investigate more SNPs in the VPr1b gene. The above observation further supports the notion that the signalling of VP may play a role in the development of a protective phenotype. The findings from these genetic association studies

specify that variations in the VPr1b gene may have a role in the development of distinct types of depression among individuals [196].

Down syndrome

Down syndrome is the prevailing autosomal trisomy, representing the most frequently encountered recognized etiology of cognitive impairment. This condition is an illness affecting many systems, characterized by a diverse array of physical traits, health issues, and developmental challenges. Individuals with Down syndrome exhibit a range of cognitive abilities. The intelligence quotient (IQ) is often measured on a scale that spans from 20 to 80. There is a suggestion that the rate of cognitive growth exhibits a deceleration as children progress in age. For instance, whereas the average IQ at one year is reported to be 70, it is projected to decrease to approximately 50 by the time they reach school age. One possible explanation for this phenomenon could be attributed to the administration of tests at varying stages of development, which may assess distinct skill sets. Specifically, it is plausible that the tests employed during later stages of development rely excessively on language proficiency.

In a study conducted by Eisenberg *et al.* (1984), a group of nine individuals ranging from 10 to 42 years of age diagnosed with Down syndrome were administered VP treatment. The researchers assessed the participants' cognitive function using word lists and visual-verbal paired related tasks. A derivative of VP was supplied over 10 days, with a daily dosage of 40 µg, using a double-blind randomized crossover design. The results of a visual-verbal paired association learning test did not demonstrate a statistically significant improvement with the administration of VP. The administration of VP did not result in an improvement in Word List memory [197].

Parkinson's disorder (PD)

PD is categorized by the impairment of various peptidergic systems in specific regions of the brain, particularly the basal ganglia (including cholecystokinin-8 (CCK-8), Met-enkephalin (MET-ENK), substance-P (SP), and Leu-enkephalin (LEU-ENK)), as well as the cerebral cortex (SRIF). However, certain peptidergic systems, such as thyrotropin-releasing hormone (TRH) and potentially VP, may remain anatomically intact in individuals with Parkinson's

disease [133]. In brain of PD patients several peptide systems appear to remain intact. The peptide concentrations in many brain regions, including the caudate nucleus, nucleus accumbens, putamen, pallidum, ventral tegmental area, substantia nigra, hypothalamus, periaqueductal grey, amygdala, red nucleus, do not exhibit significant differences compared to control samples [170]. In a comparative analysis between the Parkinsonian brain and control subjects, no significant alterations were observed in VP-like immunoreactivity within the substantia nigra, periaqueductal grey matter, locus coeruleus, and pallidum regions [171].

Autism

Autism spectrum disorder (ASD) prognosis can be linked with VP dysregulation in brain with handful of clinical evidence. The concentrations of VP in the CSF were seen to be diminished in children diagnosed with autism. Furthermore, there was a discernible correlation between symptoms observed in individuals and VP levels. Furthermore, the measurement of VP levels in the CSF of neonates has enabled the prediction of future autism diagnoses [61]. The levels of V1aR mRNA were shown to be decreased in individuals diagnosed with autism. Research conducted on the gene polymorphism of VP receptors in individuals with ASD within the Korean population revealed a noteworthy correlation between ASD and SNPs RS1 and RS3, which are located in the 50 flanking areas of the V1aR receptor [183]. The researchers have indicated noteworthy association between ASD and VP V1b receptor (VP V1bR) the polymorphism in SNPs rs28632197 and rs35369693. Parker et al. observed that i.n. administration of VP for 4-5 weeks resulted in enhanced social skills and decreased symptoms of anxiety, demonstrating the beneficial impact of VP on social behaviour in individuals with ASD [198].

Schizophrenia (SCZ)

Empirical evidence from both clinical and experimental investigations suggests that the dysregulation of VP may be implicated in the etiology of schizophrenia. The study documented by Egashira and coworkers the presence of symptoms resembling schizophrenia, characterized by impaired social behaviour, in V1aR knockout mice and VP-deficient rats. Schizophrenia based preclinical rat model generated by methylazoxymethanol acetate (MAM) prenatal

exposure exhibits reduced concentration of VP receptor in hypothalamus and prefrontal cortex [186]. Certain individuals diagnosed with schizophrenia who have abnormally elevated levels of VP in their blood exhibit symptoms such as excessive thirst (polydipsia), low osmolality (hypoosmolality), and low sodium levels (hyponatremia). The administration of antipsychotic medications exacerbates these symptoms. DDVP i.n. administration of risperidone mitigates schizophrenia related pathophysiological events. The study identified noteworthy correlations between specific variations in VP genes and schizophrenia within the 20p13 chromosomal region. These correlations were specifically observed in the 50 promoters of OT gene and rs3011589, situated in the second intron of the VP gene promoter [199].

2.3.3 Angiotensin

The origin of Renin Angiotensin System (RAS) phenomenon may be traced back to its initial identification in 1898 by Bergman and Tiegerstedt, who bestowed upon it the designation of a renal hormone [200]. RAS plays a crucial role in maintaining electrolyte balance, regulating volume of body fluid, and controlling cardiovascular function in the peripheral circulation. The enzyme renin, which is expressed in the kidney, catalyzes the hydrolysis of angiotensinogen (AGT), a precursor expressed in the liver, and results in Angiotensin I (Ang I) release with limited biological activity [201]. Angiotensin-converting enzyme (ACE), facilitates the cleavage of Ang I into the active octapeptide Ang II that plays significant role in different physiological activities. The persistent activation of RAS and elevation of angiotensin II (Ang II) levels can potentially impact angiotensin II type 1 receptor (AT1R) and can initiate inflammation, vasoconstriction, fibrosis, and augmented renal salt reabsorption [202].

Apart from above pharmacological effects, RAS exhibits neurological impacts also. The effects of brain renin have been extensively investigated and documented through a diverse array of studies and methodologies over 50 years. When Detlev Ganten postulated the existence of renin in the brain that is distinct from renal renin, it necessitated the application of rigorous evidentiary criteria [203]. Upon intracerebral administration, Ang II shows hypertensive effect and, in the presence of accessible water, prompts a consumptive response.

It was postulated that the impact of Ang II on CNS (central nervous system) was restricted to the brain due to BBB (blood-brain barrier) presence [204].

The compiled literature mainly examines RAS, with a specific emphasis on its potential implications in neurological illnesses. The literature review section subsequently engages in a discussion of the apparent disruption of RAS functions in various brain ailments, encompassing mental illnesses, neurodegenerative, neuroinflammation, disorders, and brain deformities. Apart from that preclinical, clinical and intellectual testimonies pose a potential of Ang in neurological disorders.

2.3.3.1 Pharmacology of Angiotensin

RAS is a complex system with multiple regulatory mechanisms. It serves crucial functions in renal and cardiovascular physiology, primarily by modulating blood pressure (BP) and maintaining hydroelectrolyte balance. The role of RAS in the CNS holds significant physiological importance. The various brain regions within the BBB of mammals have been found to include all the necessary elements of the RAS (Figure 2.3). Nevertheless, a recent investigation has proposed that cerebral Ang II is derived via the uptake of Ang II from the bloodstream rather than being locally produced within brain [205]. AGT serves as a paternal precursor peptide within the RAS, from which other angiotensin peptides are derived. Numerous empirical studies have provided substantial evidence supporting the existence of AGT molecule, which consists of 485 amino acids, within the brain. However, the specific biological origin of this molecule remains undetermined. Multiple studies have documented that a majority exceeding 90% of AGT peptides are generated inside astrocytes. Additionally, there have been reports indicating that the synthesis of these peptides also occurs to some extent in glial and neuronal cells [206].

However, a limited number of researches have documented that the regulation of hypertension and fluid balance in rats and mice is influenced by AGT modulation. The observed phenomenon can be elucidated by considering the location of AGT inside the brain regions that are responsible for regulating circulatory activities [207]. Ang I, a peptide with little physiological activity, has been detected in the brain, albeit in relatively low concentrations.

Ang II presence and its corresponding receptors in the brain are also well documented in referred testimonials. Moreover, it has been observed that the expression of AT1R is abundant in the brain, but the expression of AT2R is limited and comparatively lower. The AT1R primarily mediates Ang II detrimental effects, whereas the AT2R opposes the actions of AT1R. The administration of Ang II inside cerebral region leads to AT1R activation, hence controlling over activity of brain's RAS. The activation of AT1R leads to cellular damage, which is facilitated by the activation of JNK and MAPK signalling pathways [208]. Ang II modulates brain neurophysiology, especially cardiovascular centres. This effect mediates Ang II interaction with GABAergic as well as glutamatergic neurons in hypothalamus [209]. In an investigation, one researcher connected angiotensin peptide to AD management [210].

Ang II activation through AT2R pathway was found to promote an augmentation in brain size and the growth of neural connections. Ang II can undergo enzymatic conversion into Ang III (Angiotensin III). This conversion is facilitated by the enzyme amino peptidase M (AMN), which acts by removing aspartic acid residue located on Ang II amino terminal. Furthermore, it has been empirically shown that Ang III has a binding affinity towards AT1R, AT2R, and non-AT receptors. Ang III has been demonstrated to possess significant relevance in BP regulation. Ang III can undergo enzymatic conversion by AMN, resulting in Ang IV (Angiotensin IV) genesis [211]. However, Ang II can directly generate Ang IV through the catalytic activity of dipeptidyl amino peptidase. Angiotensin IV exerts its effects via binding to AT4R, hence facilitating neuroprotective mechanisms [212].

The primary site of Angiotensinogen production is the liver, where it undergoes hydrolysis by renin. This hydrolysis results in the formation of a decapeptide known as Ang I, which consists of the amino acid sequence Asp^{1}-Arg^{2}-Val^{3}-Tyr^{4}-Ile^{5}-His^{6}-Pro^{7}-Phe^{8}-His^{9}-Leu^{10}. Ang I undergo conversion by ACE (Angiotensin Converting Enzyme). This enzymatic process results in Ang II genesis, an octapeptide with biological activity. Ang II is Asp^{1}-Arg^{2}-Val^{3}-Tyr^{4}-Ile^{5}-His^{6}-Pro^{7}-Phe^{8}. Ang II effects facilitate GPCR that possess ATIR and AT2R [213]. In recent decades, there has been a delineation of novel constituents within the RAS. A homologous enzyme to ACE, known as ACE2, was identified and demonstrated to cleave the

C-terminal phenylalanine residue from Ang II, resulting in the formation of Ang (1-7) is (Asp^1-Arg^2-Val^3-Tyr^4-Ile^5-His^6-Pro^7).

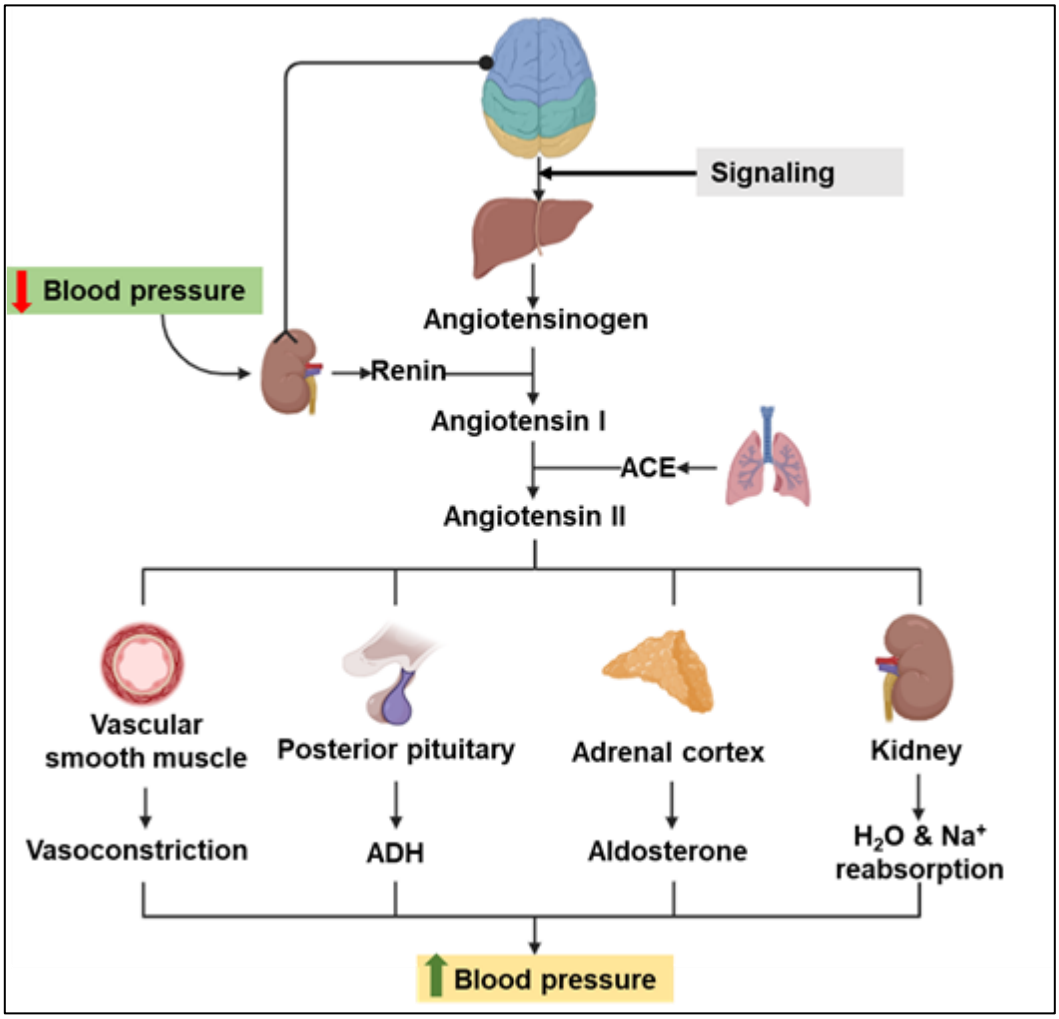

Figure 2.3: Signalling mechanism of angiotensin in body

Ang-(1-7) can be synthesized through the enzymatic actions of PEP (Prolyl Endopeptidase) and NEP (Neutral Endopeptidase) on Ang I. The aforementioned heptapeptide functions as a ligand for the GPCR-Mas, which exhibits expression in brain, heart, kidney, and blood vessels. In one study, two compounds, Ang A (Ala^1-Arg^2-Val^3-Tyr^4-Ile^5-His^6-Pro^7-Phe^8) and Alamandine (Ala^1-Arg^2-Val^3-Tyr^4-Ile^5-His^6-Pro^7), were introduced into the existing complex system. Angiotensin A can be generated through the process of decarboxylation of the Asp1

residue to Ala1 from Angiotensin II. Its biological activity is mediated by binding to the particular AT1R [214]. See Figure 2.3 for pictorial representation. In contrast, Alamandine, a peptide that specifically relates with the MrgD (Mas-related-G protein-coupled receptor), is produced through enzymatic activity [215].

Extensive research has been conducted to identify and investigate many RAS components within brain, particularly about its role in neuroprotection [216]. The excessive activation of classical axis, which involves ACE-Ang II and AT1 receptors, within CNS has been associated with various detrimental effects. These effects include cerebral blood flow decrease, occurrence of neuroinflammation, heightened oxidative stress, and impaired cognitive functions. In contrast, the alternate RAS axis, comprised of Mas and ACE2-Ang-(1-7) receptors, elicits neuroprotective effects [217]. Mogi *et al.* observed that the activation of Ang II signalling through AT1R resulted in neurotoxic effects and disruption of BBB [218]. Activation of Ang II pathway through AT2R receptor was found to promote an augmentation in brain size and growth of neuronal system. Ang IV can also be produced directly from Ang II through the enzymatic activity of dipeptidyl amino peptidase. Angiotensin IV exerts its effects via interacting with the AT4R, hence facilitating protective mechanisms [219].

In the brain, affinity of pro-renin for pro-renin receptors (PRRs) is higher than renin, resulting in cleavage of AGT. Therefore, the activation of angiotensin receptors through stimulation of renin/pro-renin signalling leads to cognitive impairment [220]. To establish the existence of a brain RAS, it was necessary to employ neurophysiological techniques. Researchers observed that Ang II-induced activity in neurons was effectively blocked by the administration of saralasin, an Ang II antagonist [221]. Expanding upon the notion, it was postulated that if Ang II, which is produced by cerebral renin, had a role in hypertension generation, then the suppression of cerebral RAS activity would result in a BP reduction.

The administration of saralasin as a sole intervention within the cerebral region of stroke-prone hypertensive rats (spSHR) resulted in a transient reduction in systemic hypertension. This phenomenon was observed even in cases when kidney renin was absent, as demonstrated by bilateral nephrectomy performed on spSHR rats [222]. Subsequently, the veracity was

substantiated by employment of molecular inhibitory techniques targeting Ang II genesis in CNS. The application of immunocytochemistry utilizing renin antibodies revealed its presence in neuronal cells. The activity related to renin was observed in pituitary, hypothalamus, and pineal glands. The utilization of antibodies targeting Ang II has provided valuable insights into its localization inside the hypothalamus and CVOs, with a particularly pronounced presence observed in spontaneously hypertensive rats (SHR) [223]. The brain Ang II levels were elevated in comparison to levels of Ang II present in circulation, indicating a potential dissociation between the two systems. After establishing brain Ang II presence, the subsequent step in substantiating this claim necessitated the demonstration of its endogenous synthesis within the brain [224]. The application of molecular biology techniques facilitated the quantification and spatial determination of messenger RNA (mRNA) molecules encoding for AGT and renin within the brain. Stornetta and colleagues employed the technique of in situ hybridization mRNA to localize the synthesis of AGT to glial cells [225].

2.3.3.2 Pharmacokinetics of Angiotensin-II

Following an intravenous infusion of angiotensin II in adult patients, the blood concentrations of angiotensin II observed were similar to those at baseline. After three hours of treatment, the serum level of angiotensin I the precursor peptide for angiotensin II decreases by nearly forty percent. In plasma, erythrocytes, and various major organs (including the gut, lung, liver, and kidney), aminopeptidase A and angiotensin-converting enzyme 2 metabolize it respectively. Approximately 40% of the activity mediated by the angiotensin II type 1 receptor (AT1) is associated with angiotensin III; the activity related to aldosterone production is somewhat akin to that of angiotensin II. Ang-(1-7) promotes vasodilation and exhibits effects opposite to those of angiotensin II on AT1 receptors. Nevertheless, the official prescribing guidelines explicitly state that no formal studies on the metabolism of angiotensin II have been conducted.

2.3.3.3 Preclinical applications of RAS in neurological disorders

Alzheimer's disease (AD)

Cognitive decline is connected with shortfalls in cerebral blood flow, which have adverse consequences for the outcome of ischemia. Cerebral hypoperfusion is closely linked to the

deterioration of cognitive function observed in both ageing process and vascular dementia. Ang-(1–7) administration has been found to protect against cognitive impairment in rats that have been exposed to cerebral hypoperfusion. The observed neuroprotective effect was found to be linked with an elevation in formation of nitric oxide (NO), a reduction in neuronal loss, and a suppression of astrocyte proliferation, specifically in hippocampus [226]. The findings indicate that a loss in ACE-2 leads to compromised cognitive performance, potentially due to heightened oxidative stress and a reduction in BDNF (brain-derived neurotrophic factor). Table 2.6 highlights the major preclinical studies related to Angiotensin.

Studies conducted on rats treated with ACE inhibitors (ACEi) have yielded inconsistent findings on the impact on amyloid-beta (Aβ) levels in the brain. Although the precise mechanism remains uncertain, there is substantial evidence suggesting that therapies based on the RAS could be highly beneficial in the treatment of AD. The levels of Ang-(1–7) were observed to be decreased in both hippocampus and cerebral cortex of a mouse model representing sporadic AD. This reduction in Ang (1–7) levels was found to be correlated with hyperphosphorylation of tau [227]. In a study examining the effects of Ang (1–7) treatment on cognitive deficits induced by diabetes, it was observed that this treatment mitigated cognitive impairments and improved the integrity of hippocampal synapses at the ultrastructural level. Additionally, marked reduction in levels of Aβ oligomers, soluble Aβ42, and insoluble Aβ40 provided substantial anti-alzheimer activity. The observed protective effects were shown to be significantly attenuated when the Mas receptor antagonist was co-administered, suggesting that the Mas receptor mediated the effects [228].

Table 2.6: Preclinical studies of Angiotensin for neurological disorders

S.No.	Neurological disorder	Preclinical studies & Model	Inference
1.	Alzheimer's disease	Chronic cerebral hypoperfusion induced model in Male wistar rats & administered with Ang-(1–7) using ICV	Neuroprotective effect was found to be linked with an elevation in the formation of nitric oxide, and a suppression of astrocyte proliferation, specifically in the hippocampus.
2.		Male SAMP8 mice (Sporadic AD) and Male P301S transgenic mice (Expression of microtubule-binding protein tau human based)	Hyperphosphorylation in brain
3.	Parkinson disease	MPTP	Administration of AT1R antagonists or ACEi demonstrates a noteworthy reduction in neuronal cell death
4.		Haloperidol induced dyskinesia murine model	Neuroprotective impact of AT1R antagonists is linked to the creation of heterodimers consisting of AT2R/ AT1R
5.	Stroke	Cerebral ischemia in male Wistar model and ICV Ang-(1–7)	Reduced iNOS expression, as well as the downregulation of various proinflammatory cytokines such as IL1α, chemokine receptor type 4, IL6.
6.	Depression	Forced swimming test-based model in Male CD-1 mice	Captopril predominantly reduced depression effects
7.		Shock induced model in Male wistar rats	Perindopril mediates antidepressant effects verified via learning helplessness
8.		Forced swimming test in male albino mice	Losartan reported antidepressant effect with single dose subcutaneously administered 20 mg/kg
9.		Unpredictable chronic mild stress model induced in Male C57BL mice	Valsartan reported antidepressant effect when studied via forced swimming test, tail suspension test, novelty suppressing feeding test and sucrose preference test

S.No.	Neurological disorder	Preclinical studies & Model	Inference
10.		Unpredictable chronic mild stress model induced in swiss albino mice	Irbesartan (Dose: 40 mg/kg via per oral for 4 weeks) linked antidepressant effects verified using forced swimming test and tail suspension test
11.		Forced swimming test-based model in Male wistar rats	Losartan (Dose: 45 mg/kg; single intraperitoneal administration) exhibit antidepressant effects
12.		Forced swimming test-based model in Male wistar rats	Losartan (Dose: 10 nmole/side; ventral hippocampus administration) exhibit antidepressant effects
13.		LPS induced model with 1 mg/kg dose continuous for 7 days in Male wistar rats	Losartan (Dose: 3 mg/kg; intraperitoneal administration for 24 days) exhibit antidepressant effects
14.		LPS induced model with 0.5 mg/kg dose continuous for 7 days in Male SD rats	Candesartan (Dose: 1 mg/kg; Peroral administration for 2 weeks) exhibit antidepressant effects
15.	Anxiety	Rats (Lister Hooded; male) studied for social test, elevated plus maze test	Captopril (Dose: 1/10/50 mg/kg; intraperitoneal administration) exhibit anxiolytic
16.		Male wistar rats studied for elevated plus maze test	Losartan (Dose: 0.001/0.01/1 mg/kg; per oral administration) exhibit anxiolytic
17.		Male wistar rats studied for elevated plus maze test	Losartan (Dose: 2 nmol; intracerebral administration) exhibit anxiolytic
18.		LPS induced model with 50 µg/kg dose; intraperitoneal in spontaneous hypertensive rats	Candesartan (Dose: 1 mg/kg; sub cutaneous administration) anxiolytic activity
19.		Ang-(5-8) induce wistar male rats	Valsartan (Dose: 100, 400, 1000 and 10000 pmol/µL directly administered into brain) as anxiolytic
20.		LPS induced model with 250 µg/kg dose; intraperitoneal in SD male rats	Candesartan (Dose: 1 mg/kg; per oral administration) anxiolytic activity

Parkinson disease (PD)

The occurrence of PD is linked to dopaminergic neurons, and its degeneration in substantia nigra region especially pars compacta. The gradual deterioration and impairment of several neurotransmitter systems, both classical and nonclassical, such as RAS, accompanies disease progression [229]. The aggregation of α-synuclein, which contributes to microglial activation in PD, is linked to the upregulation of AT1Rs and activation of NADPH oxidase [230]. AT1Rs inhibition through the administration of candesartan and telmisartan effectively mitigates the adverse consequences associated with α-synuclein in dopaminergic and microglia. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) injection in rodent model with administration of AT1R antagonists or ACEi demonstrated a noteworthy reduction in neuronal cell death. Comparable outcomes were achieved in cultures of primary mesencephalic cells [231].

Additionally, ACEi or AT1R antagonists beneficial effects are achieved by inhibiting microglial and NADPH activation, as well as proinflammatory effects and prooxidative suppression mediated by cytokines. Subsequent investigations have revealed that the administration of candesartan does not exert an impact on motor efficiency or the levels of dopamine and serotonin in the striatum. However, it does result in an upregulation of D1 & downregulation of D2 receptors. In preclinical studies of rat models, neuroprotective impact of AT1R antagonists is linked to the creation of heterodimers consisting of AT2R/ AT1R. It is noteworthy to mention that the treatment of lisinopril and candesartan has decreased pro-inflammatory (IL-1, TNF-α) release and glutamate haloperidol dyskinesia murine model [232]. This particular condition is classified as a dopaminergic disorder that arises from the impairment of striatal neurons. Significantly, the utilization of an ATR1 antagonist, hypothesized to exert its mechanism of action via glial cells, demonstrates considerable efficacy in preserving neuronal health and preventing the demise of dopaminergic cells in a murine model of PD. Dopamine has been observed to limit the activation of microglial RAS by modulating the ATR1/ATR2 ratio in these cells. Apart from that free Ang (1-7) effectively reduce levels of NADPH and cytokines. This modulation results in the suppression of the pro-inflammatory path of RAS, as demonstrated in in vitro cell cultures [233].

Stroke

Increased activity of the AT1R is linked to cerebrovascular disease associated with hypertension. The excessive ACE/Ang II/AT1 receptor axis activation is believed to have a role in development of acute ischemic stroke. This is due to its ability to constrict cerebral blood vessels and profibrotic and proinflammatory effects in the brain tissue [234]. Sumners in initial findings indicates therapeutic benefits of Ang-(1–7) in mitigating neurological impairments and CNS damage resulting from stroke, especially in cerebral ischemic. In the cerebral ischemia murine model, cerebral artery blockage demonstrated that injection of Ang-(1–7) activates ACE2 resulted in a considerable reduction in cerebral infarct size and improvement in neurological impairments, as after 72 h of administration. The researchers above have demonstrated that administering Ang-(1–7) orally after a stroke reduces cerebral damage and enhances neurological functions in rats. Ang-(1–7) protective effect is facilitated through activation of Mas receptor. This mechanism involves suppression of iNOS expression, as well as downregulation of various proinflammatory cytokines such as IL1α, chemokine receptor type 4, and IL6 [235].

Additionally, the reduction in microglial activation is indicated by the decreased expression of CD11b, a marker associated with macrophage/microglial activation. In stroke, blockage of middle cerebral artery resulted in high BP [236]. Additionally, there was an increase in signaling of CCR2 (chemokine receptor 2) and MCP-1 (monocyte chemoattractant protein-1), along with presence of neuroinflammation in the RVLM (rostral ventrolateral medulla), which is a brain stem region and regulates BP. Furthermore, the pressor response induced by stroke and signaling of MCP-1/CCR2 stimulated by stroke was abolished with the administration of Ang-(1–7) into RVLM. Ang-(1–7) administration resulted in lowering of oxidative stress levels and a suppression of NF-κB activity [237]. Additionally, this treatment led to a reduction in microglial cell count in the striatum, suggesting a potential mitigation of cerebral inflammation and a propensity for increased neuronal survival within the same region. The observed protective effect was found to be associated with Mas receptor activation [238].

Schizophrenia (SCZ)

Schizophrenia is a highly debilitating psychiatric disorder characterized by intricate factors contributing to its development and progression. The involvement of RAS in schizophrenia etiology has been proposed due to its direct modulation of GABA, glutamate, inflammation, dopamine, peroxisome, all of which have been connected with development of schizophrenia [239].

Furthermore, previous studies have indicated that ACE exhibits an interaction with dopaminergic processes inside the brain, hence influencing the modulation of prepulse inhibition in mice [240]. This particular behavioral measure is often regarded as gold standard in animal studies investigating schizophrenia. The presence of immune-inflammatory reactions has been documented in individuals with SCZ and has been linked to the advancement of disease and cognitive deficits. It is worth mentioning that there exists a noteworthy and substantial positive linkage between ACE activity and the levels of IL-17a and IFN-γ in individuals diagnosed with SCZ [241].

Depression

Preclinical studies effectively paved the way for RAS dominance in depression. In 1989, Giardina studied Captopril's antidepressant effects using forced swimming test-based model in male CD-1 mice [242]. In 1990, Martin and coworkers studied Perindopril, a prominent ACE inhibitor with antidepressant efficacy in shock based male wistar rat model [243]. Similarly, in 1999 Gard and coworkers reported AT1 blocker i.e., Losartan antidepressant effects in male albino mice using forced swimming test [244]. In 2014, Ping and coworkers reported Valsartan another AT1 blocker in male C57BL mice induced by unpredictable chronic mild stress model and verified using forced swimming test, tail suspension test, novelty suppressing feeding test and sucrose preference test [245]. In 2017, Ayuub and coworkers studied Irbesartan linked antidepressant effects in Swiss albino mice induced by unpredictable chronic mild stress model and verified using forced swimming test and tail suspension test [246]. In 2018, Diniz and coworkers reported two research methodologies about Losartan exhibiting antidepressant effects in Male wistar rats when administered with intraperitoneal and ventral hippocampus [247]. Similarly, Salmani reported antidepressant effect of Losartan

in lipopolysaccharide (LPS) depressant model in male wistar rats [248]. In 2019, Gong reported Candesartan antidepressant property in LPS induced male wistar model [249].

Anxiety

Several preclinical evidences paved the way for RAS in mitigation of Anxiety. In 1990, Costall reported the anxiolytic activity of Captopril in rats (Lister Hooded; male) when studied using social test, elevated plus maze test [250]. Similarly, Kaiser *et al*, in 1992 reported Losartan (AT1 blocker) prominent anxiolytic activity when studied via elevated plus maze test in male wistar rats [251]. Continuing this hypothesis, Braszko in 2003 reported anxiolytic activity in male wistar rats when Losartan was intracerebral administered to them [252]. In 2011, Benicky reported Candesartan anxiolytic activity in LPS induced spontaneous hypertensive rats [253]. Lopez and coworkers studied, Losartan anxiolytic activity by direct amygdala administration in male rats [254]. Ping *et al*, in 2014 reported Valsartan anxiolytic activity in C57BL/6J male mice [245]. Genaro in 2017 reported Losartan (directly administered into brain) as anxiolytic in Ang-(5-8) induce wistar male rats [255].

2.3.3.4 Clinical applications of RAS in neurological disorders

Alzheimer's disease (AD)

AD is a neurological illness that is allied with aging and has a prevalence rate of 5-10% among those who are 65 years or older. Dementia manifests as a significant sign categorized by the existence of memory loss, accompanied by a gradual decline in cognitive functioning and alterations in personality. Two ideas have been postulated to elucidate the pathophysiology of AD. The amyloid β (Aβ) hypothesis posits that the generation of Aβ is a result of proteolytic activity carried out by the β- and γ-secretase enzymes during the processing of APP [256]. The presenilin theory is supported by evidence suggesting that it can lead to neurodegenerative and behavioral effects that are not dependent on amyloid precursor protein (APP) [257]. There are several consequences associated with decreased activity of Ang II, including BP reduction, acetylcholine reduction, and an increase in substance P levels. Substance P is a substrate of ACE and has been found to enhance the activity of neprilysin, that degrades amyloid β [258]. Table 2.7 highlights the major clinical studies related to RAS.

Role of RAS in AD was proposed based on the elevated serum angiotensin-converting enzyme (sACE] activity in deceased AD patients when compared to a control group without AD. Both Ang III and Ang II were markedly elevated in brain tissue obtained from the midfrontal cortex of individuals with AD in comparison to age-matched control subjects after post mortem. Notably, the findings indicated a strong correlation between Ang III, rather than Ang II, and the accumulation of Aβ and tau proteins [259].

National Alzheimer Coordinating Center database consisted of 29 AD centers with a total sample size of 890 males in US. The brains of hypertension patients who received ARBs exhibited reduced levels of Aβ deposition indicators in comparison to those who were treated with other antihypertensive drugs. The study utilized a database from the U.S. Veteran Affairs, consisting of a sample size of 819,491 individuals. The findings suggested that ARBs use was linked to a noteworthy decrease in both the occurrence (55% reduction) and advancement (70% reduction) of dementia and AD. This reduction was observed when comparing ARBs to the ACE inhibitor lisinopril, as well as other cardiovascular medications [260]. A research group in Taiwan has provided evidence indicating that ARBs use is allied with a decreased risk of evolving dementia. The use of ARBs has been found to potentially decrease the likelihood of developing dementia in those with a high risk of vascular complications. Patients treated with higher ARBs doses had a greater degree of protection against dementia and its various forms. ARBs have demonstrated efficacy in preventing AD, irrespective of their specific pharmacological class [261].

Additionally, the effects of ACEi may be similar to those of ARBs. Though, the benefits vary depending on the drug classes used to treat cognitive impairment in aging. Specifically, the usage of ARBs (angiotensin receptor blockers) may be more active than ACEi in lowering the occurrence of cognitive impairment in aging individuals. Phase II multi-center RADAR (Reducing Pathology in Alzheimer's Disease through Angiotensin Targeting) trial, which is based in the United Kingdom and aims to recruit a total of 228 participants. This trial focuses on comparing the effects of losartan with placebo in individuals with Alzheimer's disease who have either hypertension or normal blood pressure [262]. A Phase II experiment, known as SARTAN-AD, will be conducted to assess perindopril and telmisartan safety in AD and

hypertensive patients. The trial will focus on evaluating the similarities in design and size between the two treatment options. The initial pilot Phase, I of the HEART trial, consisting of 66 participants, aims to examine the impact of two different dosages of telmisartan compared to a placebo on the levels of RAS components in the cerebrospinal fluid of African Americans with prone to AD. A study of comparable size, consisting of 72 participants, known as the CEDAR study, will examine and compare the impact of candesartan and placebo on various cardiovascular indicators in individuals with mild cognitive impairment [263].

The observed reduction in ACE2 activity was found to be linked with elevated Aβ levels and phosphorylated tau. The ratio of Ang II: Ang (1-7) in midfrontal cortex was shown to be higher in individuals with AD compared to a control group of individuals of same age [264]. Specifically, levels of Ang II were significantly elevated, whereas levels of Ang (1-7) remained unaltered. In AD, there is an observed increase in the ratio of Ang II to Ang-(1–7), which serves as a proxy measure for ACE2 activity. Moreover, it was shown that individuals with AD had a notable decrease in the plasma levels of Ang-(1–7) in comparison to a control group that was carefully matched. This finding implies that measuring plasma Ang-(1–7) could serve as a promising biomarker for diagnosing AD [226].

Parkinson disease (PD)

Both ACE and ACE2 have an impact on dopamine and are reciprocally influenced by it. The literature has demonstrated that the reduction in dopamine levels leads to an upregulation of Ang II expression [265]. Consequently, this upregulation can either promote or hinder dopamine synthesis and facilitate dopamine release via modulating the activity of ATR1 and ATR2 receptors. The ATR1 molecule can exert allosteric inhibition on the activation of the dopamine D1 receptor [266]. A reduction in the number of ARBs has also been observed in striatum and substantia nigra of deceased individuals with PD, as evidenced by post-mortem brain examinations. The activation of glial cells is linked to the death of dopaminergic cells in PD, and there is a notable increase in RAS activity within these cells among individuals with PD [267].

Table 2.7: Clinical studies of Angiotensin for neurological disorders

S.No.	Neurological disorder	Clinical studies	Inference
1.	Alzheimer's disease	Assessment of sACE activity	Elevated sACE activity levels when compared to a control group of individuals without AD
2.		Post mortem brain tissue of individuals with AD in comparison to age-matched control subjects	Ang III and Ang II were markedly elevated in AD patients in comparison to control subjects
3.		Database of 890 males brain autopsy from 29 AD centers.	Brains of hypertension patients who received ARBs exhibited reduced levels of Aβ deposition indicators in comparison to those who were treated with other antihypertensive drugs.
4.		Database from the U.S. Veteran Affairs, consisting of a sample size of 819,491 individuals.	Use of ARBs was linked to a noteworthy decrease in both the occurrence and advancement of AD and dementia. This reduction was observed when comparing ARBs to the ACE inhibitor lisinopril, as well as other cardiovascular medications.
5.		RADAR	ARBs are more effective than ACEi in lowering the occurrence of cognitive impairment in aging individuals.
6.		Phase II experiment as SARTAN-AD	Data awaited
7.		Initial pilot Phase I of the HEART trial	Data awaited
8.		CEDAR	Data awaited
9.		CALIBREX study aims to investigate and compare the effects of cognitive impairment and hypertension in post mortem brains.	Higher ratio of Ang II:Ang (1-7) in AD patients
10.	Parkinson' disease	Post mortem of Parkinson's disease brain individuals	Reduction in the number of ARBs has also been observed in individuals with PD, as evidenced by post-mortem brain examinations.
11.	Stroke	HOPE	Findings revealed a 32% decrease in Stroke when ramipril was administered
12.		PROGRESS	Observed reduction in recurrence rates ranged from 5% to 43%. As per the findings of the PROGRESS investigators, it is anticipated that a reduction of 10 mm Hg in systolic BP will result in a modest

S.No.	Neurological disorder	Clinical studies	Inference
			decrease of 28% in the likelihood of stroke recurrence within this particular cohort.
13.	Schizophrenia	Patients with either the I/D or D/D genotype	Finding suggests that the ACE functional polymorphism may be considered a risk factor for several psychiatric illnesses and potential association between specific antihypertensive medications, namely the ACE inhibitor captopril and the ARB losartan, and an increased susceptibility to SCZ and psychotic episodes
14.	Depression	1805 patients suffering from hypertension and depression	Patients suffering with both of these disorders reported high renin levels
15.		Patients prescribed with ACE and ARBs inhibitors employed for hypertension management	Patients represents healthy mental life due to AT1 and ACE receptor genes association with emotional diseases, while ACE gene polymorphic association with senile linked depression and histopathological examinations of these patients depicted smaller frontal gyri and larger temporal regions of brain.
16.		Polymorphic gene comparison studies were done in females in respect to ACE gene when prescribed with SSRI or tricyclic antidepressants	Patients reveal high and speedy antidepressant effect
17.		Candesartan administration to patients with depression and diabetes type 2	Patients reported enhance scores for depression response
18.	Anxiety	Angiotensin linked genes (ACE, Ang, AT1) in men diagnosed with panic disorder	No significance difference in AT1 and Ang receptor genes was observed, but ACE gene reported polymorphic changes in significant manner in terms of enhanced levels of ACE in serum and Ang II
19.		Comparison of patients with PTSD prescribed with ARBs or ACE inhibitors and Ca^{2+} channel or β blockers	Low stress symptoms in PTSD patients prescribed with ARBs or ACE inhibitors
20.		Patients' neuroimaging in relation to amygdala stimulus to fear	Losartan administration decrease the anxiety response

Stroke

The CAPPP (Captopril Prevention Project) randomized study provided evidence indicating that the administration of captopril, as opposed to diuretics or β-blockers, resulted in a significant decrease in the incidence of stroke, with a reduction rate of 25%. The HOPE (Heart Outcome Prevention Evaluation) experiment was carried out on a cohort of 9297 patients who had vascular disease or diabetes, together with an extra vascular risk factor. These patients were monitored for 4.5 years. The study findings revealed a significant reduction in the overall risk of stroke by 32% when the ACE inhibitor ramipril was administered [268]. The LIFE (Losartan Intervention for Endpoint reduction in hypertension) randomized trial demonstrated a notable discrepancy in stroke incidence between losartan and atenolol in a sample of 9193 individuals aged 55-80 years with essential hypertension. Despite comparable reductions in blood pressure, losartan exhibited a more favourable outcome in terms of stroke rate [269]. In PROGRESS (Perindopril Protection Against Recurrent Stroke Study) examination for the efficacy of an ACE inhibitor in preventing the recurrence of stroke in individuals who had previously experienced a stroke. The research was conducted using a sample size of 6105 individuals. The findings indicated that the administration of the ACE inhibitor perindopril, either alone or in conjunction with indapamide, reduces stroke recurrence in both hypertensive and normotensive patients. The observed reduction in recurrence rates ranged from 5% to 43%. As per the findings of the PROGRESS investigators, it is anticipated that a reduction of 10 mm Hg in systolic blood pressure will result in a modest decrease of 28% in the likelihood of stroke recurrence within this particular cohort [270].

The SCOPE trial, which involved a sample of 4937 senior hypertension patients, demonstrated that administration of candesartan resulted in a 28% reduction in non-fatal stroke occurrences. The findings of ACCESS trial, which stands for Acute Candesartan Cilexetil Therapy in Stroke Survivors, indicate that use of an angiotensin receptor blocker (ARB) is a safe approach during hypertensive acute phase in individuals who have experienced a stroke. Furthermore, the study suggests that ARB treatment may lead to a reduction in death rates, regardless of its impact on blood pressure regulation. There is a lack of antihypertensive trials in the context of secondary stroke prevention [271]. The study conducted in Taiwan involved a population-based follow-up, with a total of 5445 people who were included based on Taiwan's National Health Insurance

data. The study's outcomes advocate that ARBs may be measured as a first choice for antihypertensive therapy in patients with both hypertension and diabetes, to prevent strokes. The group receiving ARBs demonstrated a 26% reduction in stroke incidence compared to group receiving ACEi [272]. Collectively, many trials have demonstrated that administration of an ACEi or an ARBs may yield a favourable effect on occurrence of stroke. Clinical and experimental observations support the concept that AT2 receptors may influence stroke. Consequently, it is posited that ARBs may be more effective in the prevention of stroke [273].

Schizophrenia (SCZ)

According to a meta-analysis study, there exists a correlation between decreased levels of ACE gene expression and elevated susceptibility to SCZ. On the contrary, other research has demonstrated elevated ACE activity in individuals diagnosed with SCZ compared to those without the condition, particularly among carriers of the D-allele [274]. In individuals experiencing their first episode of psychosis and who have not been previously treated with antipsychotic medications, an elevation in ACE activity was observed following a two-month course of treatment with the atypical antipsychotic risperidone [275]. However, this rise was only evident in individuals with the D/D genotype subgroup. In individuals experiencing their initial episode of psychosis and having no prior exposure to drugs, the presence of the D/D genotype was found to be linked to a decrease in symptoms related to psychopathology. This association was determined by evaluating the scores obtained from the Positive and Negative Syndrome Scale (PANSS) [276].

Furthermore, patients with either the I/D or D/D genotype exhibited a more pronounced reduction in various symptoms after receiving antipsychotic medication. On the contrary, additional research has yielded findings that support the notion that possessing at least one D-allele may confer a protective effect against SCZ, either in a general sense or particularly among women. However, in a Croatian population, this polymorphism was not found to be linked to SCZ. Instead, it was observed to have a notable influence on the clinical manifestation of the illness [277]. Furthermore, a study has discovered a correlation between the ACE I/D polymorphism and the development of SCZ. This finding suggests that the ACE functional polymorphism may be considered a risk factor for several psychiatric illnesses. The studies

indicated a potential association between specific antihypertensive medications, namely the ACE inhibitor captopril and the ARB losartan, and an increased susceptibility to SCZ and psychotic episodes [278].

Conversely, RAS modulators, particularly ARBs, have shown promise as supplementary treatment options alongside existing antipsychotic medications for individuals with SCZ. The present study examined the impact of gender on inflammatory and oxidative alterations within a two-hit model for SCZ. Notably, the findings demonstrated the efficacy of the ARB candesartan in mitigating these changes in both male and female subjects [241].

Depression

Depression linked with RAS proved in preclinical implications in above section. In this section a brief discussion about clinical impressions is highlighted. In one study 1805 patients suffering from hypertension and depression reported high renin levels [279]. One of the metanalysis report discussed on patients prescribed with ACE and ARBs inhibitors employed for hypertension management tends to represent healthy mental life. The inference for this statement is on the basis of AT1 and ACE receptor genes association with emotional diseases, while ACE gene polymorphic association with senile linked depression [280]. ACE gene modulates dopamine and serotonin levels in men which play a pivotal role in elimination of depression. However, when polymorphic gene comparison studies were done in females in respect to ACE gene, where SSRI or tricyclic antidepressants reveal high and speedy antidepressant effect [281,282]. With the Candesartan administration to patients with depression and diabetes type 2 tends to enhance scores for depression response [283].

Anxiety

Clinical implications about RAS and Anxiety have been reported with well versatile reports. Olsson studied the angiotensin linked genes (ACE, Ang, AT1) in men diagnosed with panic disorder. In this study, no significance difference in AT1 and Ang receptor genes was observed, but ACE gene reported polymorphic changes in significant manner in terms of enhanced levels of ACE in serum and Ang II [284]. Khoury reported the comparison of patients with PTSD i.e., Post traumatic stress disorder prescribed with ARBs or ACE inhibitors had low stress symptoms in comparison to PTSD patients prescribed with Ca^{2+} channel or β blockers [285].

Reniecke studied the patient's neuroimaging in relation to amygdala stimulus to fear where the losartan administration decreases the anxiety response [286].

2.4 Recent advancement for enhanced neuronal delivery using various carrier systems

Treating illnesses of the CNS continues to pose challenges, mostly stemming from the limited ability of therapeutic medicines to penetrate the brain effectively. The primary constraint for the adoption of these agents is the BBB [287]. Innumerable strategies have been employed to improve the transport of substances across the BBB. These approaches encompass augmenting the lipophilicity of the substrate to facilitate passive permeability, enhancing carrier-facilitated transport across the BBB by conjugating the substrate with an endogenous uptake transporter, and reducing efflux by inhibiting transport or chemically modifying the substrate [288].

Furthermore, the utilization of nasal administration has been examined as a potential method for transporting substances to the CNS. Transporters have varied expression on the cellular surface across various cell types, and specific transporters are upregulated in certain cell types during pathological circumstances [289]. The efficacy of medication delivery could be enhanced through the utilization of targeted nano-drug delivery systems that are coupled with specific ligands. Many transporters exhibit site-specific expression, making them attractive targets for drug delivery strategies aimed at increasing absorption at specific locations or enhancing permeability across biological barriers, such as the BBB. Transporters often exhibit a wide range of substrate selectivity, in contrast to receptors, which display a higher degree of specificity towards their ligands. The observed variations can potentially yield specific benefits when considering cell-surface transporters for nano-drug delivery systems, as they give a range of options for ligand modification on the surface of nanoparticles, enabling targeted interaction with the transporters [290].

In one study, short-lived peptides for long term effects in brain regions were depicted. This strategy has been successfully tested and confirmed for RAS peptides in rat brain area especially RVLM. This approach integrates the benefits of three distinct methods, namely site-specific microinjection, liposome encapsulation, telemetry [291]. The pressor effects of Ang-(1–7) and Ang II at the RVLM exhibited an extended duration when the peptides were encapsulated, transitioning from a short duration on the order of minutes for free peptides to a

longer length on the order of days. By employing an innovative methodology, unique perspectives on the impact of Ang-(1–7) on the diurnal fluctuations of blood pressure were attained. There is a prevailing belief that this particular approach has the potential for extensive utilization across many short-lived bioactive compounds and multiple regions of the brain, hence presenting numerous opportunities for application within the realm of neurosciences. Additionally, this technology might potentially be utilized in various protocols involving several microinjections across distinct regions of the brain [292].

In the investigation of the variances between the intranasal (i.n) and intravenous (i.v) delivery of Ang II in human subjects, it was observed that Ang II concentrations were elevated in both scenarios. Nevertheless, the normalization of blood pressure occurred at a significantly faster rate with intranasal treatment. The administration of Ang II caused in a reduction in plasma norepinephrine levels and an increase in plasma vasopressin levels as compared to the IV administration group. In a subsequent investigation, Derard *et al.* observed that the administration of intranasal Ang II, in conjunction with pre-treatment of the AT1R inhibitor (valsartan), exhibited potential as a therapeutic approach for managing hypertension. These variations may be accredited to the binding of IN Ang II to angiotensin receptors in the brain [293]. However, specific investigations have demonstrated that Ang II can also induce an increase in BBB permeability through mechanisms involving tight junctions and vesicles.

2.4.1 Recent therapeutic strategies for peptide delivery

However, the clinical usefulness of these peptides is limited by the absence of efficient delivery mechanisms that deliver to the desired areas at therapeutically relevant concentrations. Drugs can be delivered at particular sites and in the right quantities using specially designed drug delivery systems, without any increasing systemic or local side effects or creating non-specific toxicity. Furthermore, the development of nanotechnology has been crucial in overcoming numerous pharmacological and therapeutic obstacles associated with peptide compounds and enhancing their clinical efficacy [12]. The fate of peptide-based treatments will depend on the possibilities of created delivery vehicles that selectively deliver them at the target site and provide more stability to peptides from the biological environment. The range of therapeutic

targets for nanoengineered lipid and polymeric-based delivery systems has expanded recently for peptide drugs to treat various medical conditions.

The most modern and efficient technique for creating therapeutic peptide analogues with the intended and targeted structures is chemical modification. Following synthesis, modifications must be made to the peptide using medicinal chemistry practices in order to replicate, stabilize, or create a model secondary or tertiary structure. This will increase the biological activity of the peptide medication and ensure its solubility, specificity, and stability.

A variety of methods are used to create stabilised peptides and proteins, including chemical synthesis, peptide modification and peptidomimetics, cyclisation, backbone and side chain modifications, peptide representing of α-helices and stabilisation, peptide imitating of β-strands and β-sheets, recombinant technology, genetic code expansion, covalent peptide/protein drug development, PEGylation of peptides and proteins, etc. [5].

2.4.1.1 Nanocarriers-based delivery approach

Nanocarriers as materials with diameters ranging from 1 to 100 nm, are frequently utilized as delivery vehicles for pharmaceuticals and diagnostic agents. They have great features, including tiny size, solubility, hydrophilicity, high selectivity, an appropriate drug-release profile. Additionally, improved permeability and retention effect (EPR) of nanocarriers aids in their accumulation in cancer tissue with leaky vasculature [294]. Many nanocarrier systems, including as lipid-based, lipid-polymer hybrid systems, polymer-based, quantum dots, carbon nanotubes, metallic nanoparticles, etc., have currently been investigated for the transport of bioactive and diagnostic chemicals [295,296].

Biodegradable and biocompatible lipids comprise liposomes, solid lipid nanoparticles and nanostructured lipid carriers (NLCs). For the administration of small-molecule drugs, diagnostic agents, peptides, proteins, nucleic acids, etc., they are the subject of extensive investigation [297]. Lipidic nanoparticles are defined by a solid lipid core sandwiched between layers of lipid membrane. High melting point lipids form a matrix that can support a greater concentration of weakly water-soluble medications in lipid-based systems, facilitating the prolonged release of the bioactive. The management of cancer and liver illnesses has been investigated by the production of electrostatic complexes with siRNA made possible by the

integration of cationic lipids into lipidic nanoparticles [298]. The FDA approved Onpattro (Patisiran), the first RNAi treatment, to treat adult hereditary transthyretin-mediated amyloidosis. It is a formulation of siRNA based on lipidic nanoparticles. Moderna and BioNTech/Pfizer recently used the potential of lipid nanoparticles to incorporate their mRNA vaccines against COVID-19 [299]. Polymeric nanoparticle systems for the targeted administration of bioactives and theranostics in the treatment of different diseases have attracted a lot of attention in the past 10 years. Biomolecules can be bonded, entrapped, or encapsulated in polymeric NPs as a drug conjugate, nanosphere, or nanocapsule, depending on the formulation technique used. Polymeric nanoparticles have several unique characteristics, including easy synthesis, chemical flexibility, low manufacturing costs, and biocompatibility (low immunogenicity, no mutagenesis) [300].

It's interesting to note that lipid-polymer hybrid nanoparticles (LPHNPs) combine the beneficial properties of lipid and polymer nanoparticles. Typically, hybrid nanostructures have a lipid shell that can be either monolayer or bilayer and a polymeric core that contains the bioactive to be conveyed. LPHNPs have decreased toxicity, improved colloidal stability, and high *in vivo* transfection efficacies. Furthermore, in certain cases, the lipid shell's façade may be coated with an exterior PEG layer or a target ligand [301]. Polymeric micelles have lately become interesting and useful drug carriers in dose form design. Usually with a size of 100 nm, polymeric micelles can readily fit poorly water-soluble medicines in their innermost core. They also show an inclination to avoid mononucleated phagocyte system scavenging [302].

Nanovesicles are nanocolloids featuring an aqueous core encased in a lipid layer, utilised for medication encapsulation in the food and cosmetics sectors. Nanovesicles are tiny structures that self-assemble naturally (e.g., exosomes) and can also be synthetically produced. Nanovesicles possess highly dynamic characteristics and are utilised for the delivery of therapeutic compounds at both topical and systemic levels. The versatility of nanovesicular structures facilitates the creation of customised drug delivery systems. In recent decades, numerous nanovesicles, including transferosomes, liposomes, ethosomes, exosomes, transethosomes, phytosomes, virosomes, niosomes, bilosomes, polymerosomes, and ufasomes, have been produced and studied for drug administration and diagnostic purposes.

Nanovesicles offer numerous advantages compared to conventional vesicles, such as superior cell membrane permeability, enhanced biocompatibility and degradability, adaptive deformation, and the capability to include both hydrophilic and lipophilic molecules. They facilitate sustained drug release, address issues of drug insolubility, instability, and degradation, enable targeted drug delivery, and improve bioavailability. The size of vesicles is extremely adjustable, ranging from micrometres to nanometres, contingent upon the materials and methods employed in their formation. Moreover, the vesicle surface can be tailored or altered using functional components to meet specific delivery objectives. The materials that compose vesicles dictate their physicochemical features, *in vivo* behaviour, and drug delivery efficacy. The criteria encompass the degradability, gastrointestinal digestibility, and intestinal absorbability of biomaterials. The administration of vesicles is versatile, allowing for intravenous, oral, transdermal delivery methods.

Polymersomes, which are innovative nanocarriers formed from amphiphilic block copolymers, exhibit remarkable potential for the precise and targeted delivery of therapeutic agents directly into cells. This potential arises from their ability to circulate in the bloodstream for extended periods, significantly reduce the degradation of macromolecules, and elicit minimal immunological responses. The unique and flexible nature of copolymers allows researchers to finely tune a variety of attributes, including size, surface characteristics, degrees of functionalization, intricate architectures, and even the capacity to respond dynamically to external stimuli [303]. In comparison to traditional liposomes, polymersomes present notable advantages, including lower mobility and higher molecular weight due to their polymer chains. As a result, polymersomes are able to form increasingly complex supramolecular structures that are characterized by tightly woven polymeric membranes. This exceptional design flexibility, in contrast to other polymeric carriers, has enabled the efficient incorporation of macromolecules within both the hydrophilic core and the surrounding membranes, significantly broadening their usage across diverse fields. Recently, there has been a focused effort to explore the construction of polymersomes as a promising platform for both diagnostic and therapeutic purposes, highlighting their potential to revolutionize treatment strategies [304].

Metallic nanoparticles (MNPs) have become a focal point of interest among investigators due to their profound inferences in the area of imaging and targeted drug administration. These tiny particles have emerged as promising alternatives for a variety of biological applications, thanks to their unique properties. Their minuscule size enables MNPs to navigate through biological and physiological barriers that are typically impenetrable to larger macromolecules, allowing them to interact with cells and tissues in innovative ways [305].

To enhance their effectiveness in medical applications, the surface properties of MNPs can be finely tuned and modified to alter their pharmacokinetic behavior, optimizing how they behave within the body. In last decade, there has been significant advancement in exploring the application of MNPs as sophisticated vehicles for drug delivery, particularly in the complex and challenging field of cancer treatment. The most widely used therapeutic strategies for combating cancer primarily involve either the application of radiation therapy or the surgical excision of tumors. In contrast to conventional therapies, the deployment of therapeutic agents provides a non-invasive method that has revealed capable outcomes in various clinical scenarios. Nonetheless, several formidable challenges remain that obstruct the full realization of this potential.

These hurdles include the difficulties in managing unwanted side effects, the incomplete destruction of malignant cells, and the limitations regarding the selectivity of the treatments available. To tackle these pressing issues, researchers are increasingly focusing on the development of intelligent drug delivery systems based on MNPs, which are designed to precisely direct therapeutic agents to the targeted cancer sites, potentially enhancing treatment efficacy while minimizing the impact on healthy tissues [306]. Carbon nanotubes (CNTs) are at the forefront of cutting-edge research in the biomedical arena, where they are exploring their potential as innovative carriers for drug delivery and sophisticated probes for bioimaging. This interest stems from their outstanding properties, which include exceptional chemical stability, remarkable optical clarity, superior electrical conductivity, and impressive thermal resilience. To facilitate precise delivery to designated cells and organs, researchers can chemically modify the surface of CNTs, incorporating a diverse array of functional groups such as bioactive proteins, nucleic acids, peptides, and pharmaceuticals. This functionalization allows for the

customization of CNTs to enhance their targeting capabilities, ultimately improving the efficacy of therapeutic interventions and imaging techniques in medical applications [307].

Quantum dots (QDs) are tiny semiconductor nanocrystals that have become highly sought after in the realm of chemotherapy due to their exceptional and distinctive properties. These remarkable quantum dots showcase a wide array of captivating optical and electrical characteristics that make them unique. Despite the rapid advancements in research and technology, their practical applications are still largely in the developmental stages. Furthermore, the extraordinary features of quantum dots including their unique physical structure, vibrant optical capabilities, and advanced electrical functionalities enable their use in a variety of innovative fields. These include medical diagnostics, tissue engineering, bio-imaging for visualizing biological processes, targeted cancer therapies, effective photothermal treatments, cutting-edge biosensing technologies, prevention measures against bioterrorism, and, crucially, facilitating the targeted delivery of drugs [308].

Lipid-drug conjugates (LDCs) are innovative pharmaceutical entities that have been structurally reformed through the covalent attachment of lipid molecules. This strategic conjugation of lipids to therapeutic agents notably enhances the lipophilicity of the drugs and alters several essential pharmacological characteristics. The resulting lipid-drug conjugates present a multitude of advantages, including improved oral bioavailability allowing for better absorption in the gastrointestinal tract customized distribution directed toward the lymphatic system, enhanced targeting capabilities for tumors, and a significant reduction in overall toxicity [309]. Low-Density Carriers have been extensively explored in a variety of research studies aimed at advancing the delivery mechanisms of gene-based therapies. In particular, lipid-siRNA conjugates have demonstrated remarkable improvements in their in vivo efficacy, showcasing the potential of these advanced formulations. Natural compounds such as cholesterol, palmitic acid, squalene, and α-tocopherol have been engaged in the creation of these siRNA-lipid conjugates. The integration of small interfering RNA (siRNA) with lipids not only helps in preventing degradation of the siRNA but also enhances its cellular uptake, leading to significantly improved efficiency in gene silencing [309].

In a detailed study, Wolfrum and colleagues investigated the effects of conjugating siRNA with a range of lipophilic substances, including various fatty acids and bile acids. Their findings revealed that this approach, which incorporated lipophilic compounds, resulted in markedly enhanced gene silencing capabilities in vivo, as well as a greater uptake of siRNA by cells, suggesting a promising avenue for future therapeutic developments [310].

The various nanotechnology-based carrier systems outlined earlier can be significantly enhanced by incorporating high surface functionalities through the use of selective ligands. This enhancement allows for more precise targeting in the delivery of cargo to specific cells and organs within the body [311]. These advanced nanocarrier systems are designed to enable highly selective and efficient absorption of the nanocarriers by diseased cells while minimizing any harmful effects on healthy cells. The following discussion will delve into a range of promising next-generation delivery technologies that have been proven effective in the targeted distribution of macromolecules, showcasing their potential applications and benefits [311]. Table 2.8 enumerates several prevalent nanotechnology-based delivery vehicles for macromolecules that have been investigated for the treatment of various illnesses.

2.4.1.2 Nanocarriers mediated delivery of peptide to brain

Drug delivery to brain

The three essential components that establish barriers between the brain's parenchyma and the surrounding cerebrovascular tissue include the blood-brain barrier (BBB), the cerebrospinal fluid-brain barrier, and the blood-cerebrospinal fluid barrier. The BBB is intricately composed of several crucial elements, such as pericytes, which support capillary stability; astrocytes, essential for neuronal health; and perivascular gaps that facilitate communication. Additionally, the glycocalyx, a dense layer of glycoproteins, along with a sturdy basement membrane, contributes to the robust architecture of the BBB, while the end feet of astrocytes encase tightly interconnected capillary endothelial cells, closely surrounded by neurons and microglia, all of which help to maintain the brain's unique environment.

Table 2.8: Recent advancements in nanocarriers mediated delivery of biomacromolecules

Peptide	Delivery system	Polymer/Material	Remarks/Outcome	Reference
MEP421(Asn–Leu–Pro–Arg acetate; brain tide)	Microspheres	Poly Lactic-co-Glycolic Acid (PLGA)	MEP421-loaded PLGA microspheres released the encapsulated peptide in a sustained way for up to 30 days. The study effectively recognized the *in vitro-in vivo* drug release association (r = 0.9746). The formulated preparation may be employed for the regulated chronic administration of MEP421 to patients afflicted with AD.	[312]
Insulin	Liposomes	Chitosan (CS)	Cationic liposomes treated with insulin demonstrated enhanced trapping of the hormone. In streptozotocin-induced diabetic mice, the formulated preparation demonstrated a significant decrease in blood glucose levels one-hour post-administration, which was sustained for up to eight hours, and was superior to both insulin solution and uncoated liposomes.	[313]
Carnosine (beta-alanyl-L-histidine)	Rod-shaped superparamagnetic iron oxide nanoparticle	Iron oxide	In the U87 human glioblastoma astrocytoma cell line, a carnosine-encapsulated nanoformulation inhibited cell growth and motility after 48 hours of administration.	[8]
Goat milk whey protein peptide	GWP-embedded liposomes (GWP-LS) and niosomes (GWP-NS)	Stigmasterol, β-sitosterol, and ergosterol,	Under various stress situations, such as NaCl concentration, temperature, and pH, GWP-NS and GWP-LS exhibited greater stability than ordinary GWP. In simulated gastrointestinal digestion, in vitro bioaccessibility, and hypoglycemic action, GWP-NS demonstrated a significant enhancement in the retention rate of GWP.	[9]

Peptide	Delivery system	Polymer/Material	Remarks/Outcome	Reference
Oxytocin	Oxytocin loaded Polymeric nanoparticles	Bovine Serum Albumin (BSA)	Intranasal delivery of OXT-nanoparticles (NP-OXTs) exhibited protective benefits against PTZ-induced hippocampus neuronal injury.	[314]
Oxytocin	Oxytocin loaded Polymeric nanoparticles	BSA	In an epilepsy mouse model, OXT-incorporated polymeric nanocarriers were discovered to be proficient of safeguarding the animal from seizure induction and re-establishing normal socio-behavioral behaviour.	[11,315]
Ovalbumin (OVA)	Mannose functionalized MWCNTs	CNTs	MWCNT-delivered OVA in bone marrow-derived dendritic cells exhibited a higher uptake in the cellular vicinity than conventional OVA, as confirmed by confocal images. Additionally, the extent of cellular uptake was significantly enhanced when MWCNT-OVA was functionalised with mannose.	[315]
CREKA	PEGylated MWCNTs (MWCNTPEG)	CNTs	The CREKA peptide was linked to the outside of the MWCNTPEG, which caused some thrombosis in the blood vessels of the tumour.	[294]

Arachnoid granulations, specialized structures extending into the venous system, house the blood-cerebrospinal fluid barrier [316]. This barrier is formed by an epithelium that is tightly bound to a basement membrane, ensuring fluid and solute balance. The interface between cerebrospinal fluid and brain tissue is layered with the pia mater a delicate membrane and a glial barrier membrane crafted from astrocytes, delineating the brain's protective borders. When arterioles transition into vessels and infiltrate deeply into the brain parenchyma, they create perivascular gaps that play a vital role in nutrient and waste exchange. Within these arterioles, the pia mater gradually evolves into a basement membrane, securing the structural integrity of this intricate system [317].

A variety of treatments are applied in distinct manners to support individuals grappling with neurological disorders, yet no ground breaking therapy has succeeded in creating an effective model capable of overcoming the selective permeability of the BBB [45]. Serving as an active interface junction, the BBB meticulously regulates the passive influx of medications and other blood-derived molecules into the brain, achieving this through a multitude of pathways such as paracellular transport, transcellular diffusion, solute carrier transport, adsorptive-mediated transcytosis, and receptor-mediated transcytosis. The primary role of the BBB is to function as a vigilant barrier that prevents the majority of substances within the bloodstream from infiltrating brain tissue and simultaneously assists in the removal of metabolic by products from the brain.

As previously discussed, the BBB is fortified by tight junctions between lipophilic endothelial cells, creating a selective barrier whereby only molecules characterized by low molecular weight and high lipid affinity are allowed passage into the brain. Consequently, the BBB poses significant challenges for the therapeutic application of larger molecules or drugs, specifically those exceeding 400 Da, as it effectively obstructs their ability to enter the brain via passive mechanisms when administered through standard routes [318]. Even though OXT acts in many ways in the brain and is involved in many neurological diseases, it is very hard to make it work as a neuroprotective drug because it can't easily cross BBB. It is very hard to make peptide-

based products that can treat neurological disorders in the brain, but it also gives us a chance to make new carrier systems that can treat CNS disorders [50]. A number of studies have been written on these fake nanoparticle drug delivery methods, focussing on the problems and unmet needs [44,297,319].

Need for Intranasal route

The foremost challenge in the field of clinical development lies in the complex task of delivering therapeutic drugs into the brain, a process hindered by the brain's limited absorption capabilities and the intricate conditions that govern drug delivery. A major barrier to achieving successful brain delivery is the formidable blood-brain barrier (BBB), a highly selective structure that serves as a protective shield, preventing a wide range of active substances from penetrating into the brain and the CNS [54]. According to a wealth of literature and published reports from peer-reviewed studies, an astonishing 98% of drug molecules designed for CNS applications are thwarted by these junctional barriers, primarily due to their specific physicochemical and biopharmaceutical properties. Within the intricate architecture of the CNS and adjacent circulating blood vessels, interstitial fluid acts as a critical obstruction, further complicating the delivery of therapeutic agents.

Moreover, the BBB is intricately linked to various transporter systems that exert additional control over the entry of drug molecules into the brain. Among these, the P-glycoprotein efflux transporter stands out as a particularly significant player, actively pumping out substances and thereby reducing their effectiveness. This limitation poses a considerable challenge, prolonging treatment durations and diminishing the therapeutic potential for numerous CNS-related conditions, including debilitating disorders such as migraines, meningitis, PD, schizophrenia, AD, and brain tumors, all of which suffer from insufficient drug availability and suboptimal therapeutic concentrations in the central nervous system.

In response to these formidable challenges, researchers have innovated a wide array of approaches and alternative pathways, ranging from invasive techniques to non-invasive strategies, all aimed at achieving therapeutic concentrations within the CNS. In the past decade, there has been a marked surge of interest in exploring the nose-to-brain delivery route, a

specialized subset of non-invasive methods designed specifically for CNS targeting. This novel approach emphasizes the utilization of the olfactory and trigeminal pathways, offering a promising solution to circumvent the significant barriers imposed by the BBB and facilitate more effective treatment of CNS disorders [55]. The benefits of nasal delivery to the brain include safety, effectiveness, non-invasiveness, lack of hepatic metabolism, ease of administration, and, most importantly, the ability to be self-administered. Drug molecules can enter the brain directly thanks to specific nasal areas. The trigeminal and olfactory regions of the nasal anatomy are examples of regions. Because of olfactory receptors, neurons, and further axon extensions into the olfactory bulb, the olfactory region has direct access to the central nervous system. One of the body's only peripheral regions thought to have direct communication with the brain is the olfactory area [54]. According to a number of pre-clinical and clinical studies, the nose-to-brain pathway functions as a possible BBB by pass and provides a non-invasive method of drug administration.

However, the nose-to-brain route only delivers 0.1% of the given amount to the CNS. The formulation bypasses the blood-brain barrier by first coming into contact with the mucosa in the nose before being delivered directly to the central nervous system. While nasal to brain transport provides an extra benefit of dose reduction, it has several drawbacks such as restricted dose administration (25–200 μL), nasal-based enzymatic degradation, and mucociliary clearance. The commercial investigation of this route has been restricted by demerit in the nose to the brain [54,56]. Alternatively, in an effort to overcome these drawbacks, scientists are constantly working to improve accessibility to this route through the development of sophisticated drugs delivery systems and the investigation of nanotechnology-based methods.

Physiology of Nasal route

Olfactory or trigeminal nerves are the routes by which smell reaches the brain. The olfactory epithelium in an olfactory part is made up of three different cell types: olfactory neuron cells, sustentacular cells, and basal cells. Olfactory neuron cells are the most important of all of these. These are embedded in the gaps between supporting cells and are also referred to as axons. They originate in the central nervous system's olfactory bulb and terminate at the apical surface

of axons [54]. Under the epithelium, there is a bed of lamina propria that has glands that make mucus and a lymphatic system with axonal bundles that connect to the nose and olfactory systems. This system brings a lot of blood and nerve cells. Filia olfactoria is made up of olfactory neurones that bind together and defend each other from glial cells. In mammals, the olfactory plexus has twenty axons and a Schwann cell from the fascicles. The area between axons, which measures between 10 and 15 nm, is a reservoir. This gap permits perineuronal transfer from the nose to the olfactory bulb in the central nervous system. The cribriform plate marks the end of the porous structure, which continues straight on to the olfactory tubercle [54,57]. From there, the thalamus, hippocampus, amygdala, anterior olfactory nucleus, prepyriform cortex, and entorhinal cortex retain the neuronal projection. Nevertheless, the exact process by which medication molecules are transported to the brain remains uncertain. Transport processes that unfold could be transcellular or involve any kind of engulfing process [54]. As of right now, no appropriate clinical standards exist that can determine the method by which this transit takes place.

Parenteral administration of OXT and other neuropeptide hormones has shown beneficial effects in neurological diseases. To improve their clinical usage, however, they must have greater bioavailability. The causes of this barrier include the peptide's size, which limits its ability to cross the blood-brain barrier, its hydrophilic nature, and its short half-life because of the presence of several enzymes, including proteases, which actively break them down [320,321]. Future advancements in the treatment of neurological illnesses in the brain may benefit greatly from the use of neuropeptide systems as medications. However, a number of peptides and their analogues are unable to effectively penetrate the BBB [322]. Due to issues with currently available traditional dosage forms, peptide-encapsulated targeted nanocarriers that can pass the blood-brain barrier were developed [323]. These targeted nanoparticles not only help peptides get to where they need to go, but they also make them more bioavailable at the right places. This makes them more effective as medicines while causing the least amount of harm to areas that aren't being targeted.

In order to transfer loaded drugs over the blood-brain barrier and to their intended locations in the central nervous system, drug delivery vehicles are essentially required. Their ability to deliver bioactives in close proximity within the CNS to provide the best possible therapeutic effect is demonstrated by their ability to modify their structure or their inherent qualities to specifically target biological targets. To carry therapeutic peptides to the brain, nanocarriers that are designed with polyethylene glycol and PEGylated dendrigraft poly-L-lysine have been used [324,325]. When using nanocarriers in long-term therapeutic contexts, these methods entail facilitating the nanostructured material's interaction with receptors or molecules to get the advantage of receptor-mediated brain penetration over the blood-brain barrier [326,327]. Transferrin (Tf) is an endogenous system that carries iron, and in the past, many exploratory investigations have tried to modify this endogenous scheme to incorporate medication formulations into the brain. Tf-anchored delivery systems may enhance drug molecule transport across the BBB since Tf-receptors are overexpressed in brain capillary endothelium. Transferrin-bound candidates can cross the BBB through ligand receptor-mediated transcytosis [328,329].

The Tf and Rabies Virus Glycoprotein ligands were anchored in PLGA and BSA polymer to create OXT-loaded targeted NPs, as reported in the recently published study. For brain delivery, abovementioned targeted nanometric drug delivery systems underwent additional *in vitro* and *ex vivo* testing. It was found that the targeting ability of created NPs indicated promise for brain delivery [330]. Additionally, OXT can penetrate the blood-brain barrier (BBB) by means of endogenous secretory RAGE (Receptor for Advanced Glycation End-Products) membrane receptor-mediated transport [44]. Many studies have been conducted on the potential of drug carriers based on nanotechnology in the treatment of different neurological illnesses; a summary of these studies is provided in Table 2.9.

Table 2.9: Nanotechnologies used so far to target the brain and treat neurological conditions

S No.	Ailments	Nanocarriers	Components of delivery system	Targeting/ ligand	Cells/animal	Outcome of the study	Ref
1.	Socio-behaviour	Polymeric NPs	Bovine Serum Albumin (BSA)	Transferrin (Tf) and Rabies Virus Glycoprotein (RVG)	BEND3 cell culture and Male Swiss-Webster mice	The results of both in vitro and in vivo studies showed that OXT-incorporated polymeric nanocarriers could move OXT across the BBB and have stronger positive effects on society.	[44]
2.	Dravet syndrome, epilepsy (Scn1a-derived epilepsy)	Polymeric NPs	Bovine Serum Albumin (BSA)	-	Male CF1 mice, 2 months old, susceptible to 6 Hz-induced seizures	In a model of epilepsy in mice, OXT- incorporated polymeric nanocarriers were able to keep the animals from having seizures and get them back to normal social behaviour.	[11]
3.	BBB permeation bioavailability through active transport and bioavailability	Polymeric NPs	Bovine Serum Albumin (BSA) PLGA	Transferrin (Tf) and Rabies Virus Glycoprotein (RVG)	Murine dendritic cells	*In vitro* tests were done on 4 unlike NPs made of 2 distinguise polymers and two different target molecules. None of the nanoformulations were found to be cytotoxic or immunogenic, and all of them showed prolonged release. The RVG-coated BSA NPs formulation was the smallest and showed the necessary in vitro release profile. This makes it the best formulation for targeting molecules in the brain and releasing them.	[331]
4.	Temporal lobe epilepsy (TLE)	Polymeric NPs	BSA	-	Pentylenetetrazole-induced epilepsy models (SD rats)	Giving OXT-NPs (NP-OXTs) through the nose helped protect hippocampal neurones from damage caused by PTZ.	[314]

S No.	Ailments	Nanocarriers	Components of delivery system	Targeting/ ligand	Cells/animal	Outcome of the study	Ref
5.	Socio-behaviour	Polymeric NPs	TRIOZAN™, a Nano biomedicine delivery platform	Trimethyl chitosan	Female & male SD rats	We checked the levels of OXT in the brain of SD rats after injecting them with PBS and TRIOZANTM nanocarriers that had already been made. When given through the TRIOZANTM platform, OXT levels in the hypothalamus were higher. It was also seen that neurogenesis got better in the lateral hippocampus.	[332]
6.	Not specified	Polymeric NPs	Poly (lactic acid)-poly (ethylene glycol)-Valine (PLA-PEG-Val) co-block polymer	-	Caco-2 cells C57Bl/ 6J male mice	In CaCo-2 cells, OXT-NPs were better at getting into the cells than free drugs. When OXT was taken by mouth, its concentration in the blood was much higher when it was encapsulated in Val-NPS than when it was free (as tested in mice).	[333]
7.	Not specified	Polymeric NPs	Chitosan	-	-	OXT-loaded chitosan nanoparticles (OXT-CSNPs) released quickly at first and then slowly over time. It was said that O-CSNPs could be a good way to deliver OXT and keep it working for a long time, which would make OXT treatment more effective while treating neurological conditions.	[334]
8.	Anxiety	Metallic NPs	Iron oxide (Fe2O3)-NPs	3-aminopropyltriethoxysilane (APTES) & Antisauvagine-30 (ASV-30) conjugated	Male SD rats	Preclinical evaluation and preparation of OXT-incorporated targeted and non-targeted iron oxide (Fe_2O_3). A potential delivery instrument for the transportation of a CRF-related peptide to the brain is represented by APTES-coated Fe_2O_3-NPs.	[335]

2.4.2 Ready to Infuse (RTI) formulation

In situations where it is critical to achieve a swift therapeutic effect or maintain a precise concentration of a drug in the bloodstream, or when medications are either unstable or inadequately absorbed through the gastrointestinal tract, intravenous infusion becomes an essential method of administration. This technique allows for the direct delivery of drugs into the venous system, effectively bypassing the complexities involved in the absorption process that occurs in the digestive system. Intravenous infusion is particularly beneficial for patients who experience severe vertigo, vomiting, or altered mental status, making oral medication intolerable. Moreover, this route is advantageous for administering drugs that could potentially cause irritation if given through alternative methods. By injecting the medication straight into a vein, it achieves rapid dilution within the bloodstream, quickly minimizing the concentration at the site of injection.

However, rapidly administering certain compounds directly into the bloodstream can lead to significant nonspecific effects on the cardiopulmonary system, which raises concerns about patient safety. As a result, healthcare providers generally avoid delivering a medication as a rapid intravenous bolus. Instead, they prefer to administer medications through a slow intravenous infusion, which takes place gradually over a designated timeframe, usually spanning several minutes, to ensure better tolerance and control of the therapeutic effect.

Following are the advantages and disadvantages of ready-to-infuse dosage forms:

Advantages

- Rapid onset of action
- Predictable mechanism of action and nearly complete bioavailability
- The gastrointestinal tract can be bypassed to eradicate the challenges of oral drug administration
- The most effective method of administration for patients who are extremely ill and comatose and are unable to ingest anything orally

Disadvantages

- Causes pain

Various therapeutic dosage forms, including peptides and anticancer agents, exhibit instability in aqueous and diluted form. Thus, these compounds are available in lyophilized and concentrated injectable form accordingly. Before being administered, these formulations must be diluted in appropriate diluents as lactate ringer's solution, dextrose, sodium chloride, or dextrose plus sodium chloride injection. The dilution of injection involves aseptic manipulation, which incorporate additional stage in delivery of medicine to patient. This is very critical when drug is delivered in emergency situation. In addition, physicochemical stability of such diluted formulation is limited up to 24 h at room temperature. These limitations create a need for ready to administer formulation of peptide molecules which can be directly administered to patient. Moreover, parenteral drug manufacturing of oxygen/photosensitive drug in plastic infusion bag becomes critical due to the semipermeable nature of infusion bag, as it allows oxygen to penetrate semipermeable layer of infusion bags. However, few infusion bags are available which are made up of materials that restrict oxygen to permeate inside the bag.

2.4.3 Conjugation of peptide molecules to enhance their pharmacokinetics and pharmacodynamic attributes

Peptides represent a type of molecular diversity that has been shown to play a crucial role in the treatment of various human ailments. Nonetheless, they represent only a small portion of the overall market when compared to conventional small molecule therapies. The combination of these two therapeutic approaches offers the potential to enhance and broaden pharmacological options while minimizing toxic effects associated with higher doses. The advantages of peptide-based pharmacology are enhanced by the use of conjugates of small molecules and peptides [336]. The drug candidate's biophysical characteristics and the resulting properties for patient use are comparable to the advancements in peptide and protein therapeutics, as the outcome is a macromolecule. As a result, the development of this form of medicinal chemistry has been more pronounced in the direction of adapting to small molecules from the large molecule perspective, rather than the alternative [336]. Increased activity, selectivity and stability are the primary advantages of peptide modification.

A rich and varied assortment of conjugates has been meticulously identified and developed across a broad spectrum of therapeutic areas, specifically designed to target an extensive range of diseases originating from endocrine, infectious, and autoimmune factors. A multitude of drug candidates have demonstrated intriguing and promising preclinical results, with a select few progressing through the rigorous approval processes to become registered pharmaceutical treatments, especially notable within the realm of cancer therapy. Among the most remarkable examples are those that take advantage of the macromolecular structure of peptides, skilfully engineered to deliver the potent effects of small molecules directly to the specific tissues where the peptide exerts its unique biological influence.

In these scenarios, the peptide not only serves as a vehicle but also enhances the pharmacological properties of the small molecule, leading to improved biological outcomes. This synergistic approach often results in reduced off-target toxicity, minimizing adverse effects on healthy tissues. Furthermore, the process of conjugating peptides can effectively address common challenges encountered in the development of small molecule drugs, especially issues related to high lipophilicity, inadequate solubility, and difficulties with cell permeability. This innovative strategy thus holds significant promise for advancing therapeutic efficacy while ensuring greater safety profiles for patients [336]. The best examples of the results of chemical modification techniques that are now being used in clinical applications include semaglutide, liraglutide, and selepressin. Certain chemical modifications, however, are unable to simultaneously restore the selectivity, activity, and stability of the proteolytic process. For example, using D-amino acid instead of L-amino acid can typically help to prolong the therapeutic peptide's plasma half-life. Peptides that have undergone D-amino acid modification, however, hardly ever show signs of biological activity [58–60]. Peptide modifications enable peptides to become more drug like and to exhibit increased activity and plasma stability.

• Advantages of peptide conjugates

A diverse array of conjugates has been identified and advanced across multiple therapeutic domains, addressing a range of disorders of endocrine, infectious, and autoimmune origins.

Numerous therapeutic candidates have demonstrated promising preclinical results, with several progressing to become licensed pharmaceuticals, particularly in the treatment of various cancers. The most compelling examples involve leveraging the macromolecular properties of peptides to target small molecule effects to tissues where the peptide exhibits biological activity. When the peptide enhances the pharmacological effects of the small molecule, the biological outcomes are improved, frequently accompanied by a significant reduction in off-target toxicity. Furthermore, peptide conjugation may effectively mitigate common formulation challenges associated with small molecule pharmaceuticals, particularly those pertaining to elevated lipophilicity, poor solubility, and limited cellular permeability [337].

• Disadvantages of peptide conjugates

Significant hurdles persist with peptide-conjugate based medicinal compounds, resulting in a higher incidence of failures than successes. The challenges are abundant, relating to both biological and chemical dimensions of the method. The latter appears more controllable and is finitely tied to biological uncertainty. Specificity continues to be a challenging objective, as it is infrequent for an extracellular target to be present in only one tissue, making the technique more appropriate for enhancing therapeutic index rather than achieving complete specificity. To augment the therapeutic index tenfold, hence facilitating a tenfold increase in dose, it is more effectively accomplished by enhancing the pharmacological action in specific tissues [338].

The rate at which these fundamental issues are resolved will significantly influence efficiency in molecular design and the prospects of forthcoming peptide-conjugate-based therapeutic candidates. Until the molecular design is more precisely delineated, individual therapeutic agents will persist in emerging by effectively overcoming existing challenges [338].

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