The biology of vasopressin

Samantha Sparapani[1,2], *Cassandra Millet-Boureima*[1,2], *Joshua Oliver*[1,2], *Kathy Mu*[1,3], *Pegah Hadavi*[1,3], *Tamar Kalostian*[1,3], *Nazifa Ali*[1], *Carla Maria Avelar*[1], *Marion Bardies*[1], *Brenton Barrow*[1], *Minky Benedikt*[1], *Giuliana Biancardi*[1], *Raminder Bindra*[1], *Lisa Bui*[1], *Zakaria Chibab*[1], *Ashley Cossitt*[1], *Jeffrey Costa*[1], *Tina Daigneault*[1], *Jocelyn Dault*[1], *Isa Davidson*[1], *Jonathan Dias*[1], *Emie Dufour*[1], *Sabine El-Khoury*[1], *Nargess Farhangdoost*[1], *Anika Forget*[1], *Alexa Fox*[1], *Myriam Gebrael*[1], *Maria Concetta Gentile*[1], *Olivia Geraci*[1], *Ansley Gnanapragasam*[1], *Elias Gomah*[1], *Elie Haber*[1], *Claudia Hamel*[1], *Thivya Iyanker*[1], *Christina Kalantzis*[1], *Sara Kamali*[1], *Elsa Kassardjian*[1], *Hryssi Krissy Kontos*, *Thi Bich Uyen Le*[1], *Daniella LoScerbo*[1], *Yan Fang Low*[1], *Danielle Mac Rae*[1], *Flore Maurer*[1], *Sana Mazhar*[1], *Alice Nguyen*[1], *Kathy Nguyen-Duong*[1], *Chelsea Osborne-Laroche*[1], *Hwi Wun Park*[1], *Emilie Parolin*[1], *Kahlila Paul-Cole*[1], *Leah Peer*[1], *Margaux Philippon*[1], *Charles-Alexandre Plaisir*[1], *Jessica Porras Marroquin*[1], *Simran Prasad*[1], *Rewaparsad Ramsarun*[1], *Saad Razzaq*[1], *Samantha Rhainds*[1], *Damien Robin*[1], *Ryan Scartozzi*[1], *Davindra Singh*[1], *Sajad Soleimani Fard*[1], *Maxim Soroko*[1], *Nastaran Soroori Motlagh*[1], *Kiri Stern*[1], *Laila Toro*[1], *Modibo Tore*[1], *Stephanie Tran-Huynh*[1], *Sarah Trépanier-Chicoine*[1], *Claudia Waddingham*[1], *Aaliyah Jasmine Weekes*[1], *Allison Wisniewski*[1] *and Chiara Gamberi*[1,*]

[1] Concordia University, Biology Department, Montreal, QC, Canada

[2] These authors contributed equally
[3] These authors contributed equally

* corresponding author:
Biology Department, Concordia University
1741 Sherbrooke St. West,
Montreal QC,
Canada
H4B 1R6
Email: chiara.gamberi@concordia.ca

Abstract

Keywords

Foreword

This review is the result of a pedagogic project at Concordia University in Montreal employing a custom "write to learn" pedagogy (Gamberi and Hall, 2019) that we have used successfully before (Selber-Hnatiw 2017, 2020). Senior undergraduate students enrolled in a Comparative Physiology course for Biology majors were taught experientially how to research and study the scientific literature and write a collaborative analytical review on the biological activity of vasopressin. We apologize to those colleagues whose important research could not cited because of space constraints.

Introduction

In human and rodents, arginine vasopressin (AVP) regulates several diverse central functions: fluid balance, blood osmolarity, reproduction, complex behavior, memory and learning. An antidiuretic hormone, vasopressin is conserved from invertebrates to vertebrates and displays species-specific amino acid (aa) changes in position 3 and 8 (Hoyle, 1999). AVP is thought to have enabled survival of land-dwelling organisms by help maintaining internal water homeostasis, blood osmolarity and tubular reabsorption in the kidneys (Boone and Dean, 2008). Because of its many physiological roles, AVP dysregulation leads to widespread complications (Tsigos et al., 2016). Here, we review the biology of AVP through examination of its normal physiological roles in kidney and heart, neurological and behavioral effects, and of how AVP dysfunction contributes to pathologies such as polycystic kidney disease (PKD) and heart failure (HF), and the pharmacological manipulation of AVP-dependent pathways in these diseases.

Early discoveries. The antidiuretic function of AVP was first demonstrated in 1913 by F. Farini in Venice and, independently, von den Velden in Düsseldorf who injected extracts from the posterior lobe of the pituitary gland into anaesthetized men to control excessive water loss

due to *diabetes insipidus* (a rare condition unrelated to type 1 diabetes) or pituitary damage (Qureshi et al., 2014; Farini, 1913; Vongraven, 1913). At the time, bovine pituitary extract was known for its oxytocic (triggers uterine contractions) and pressor (raises blood pressure) properties albeit underlying mechanisms were unknown. In 1927, such two active components were isolated and called respectively alpha- and beta-hypophamines (*i.e.*, amines driven from the hypophysis). Their chemical synthesis shortly followed and the resultant products were assigned the trade names oxytocin (alpha- hypophamine, or Pitocin) and AVP (beta-hypophamine, or Pitressin,) (du Vigneaud et al., 1954 a,b). AVP receptors (R) Avpr1a (today's V1aR), Avpr2 (V2R) and Avpr1b (V1bR) were subsequently cloned, as well as the single oxytocin receptor Oxtr (Kimura et al., 1992; Morel et al, 1992).

AVP function. AVP secretion from the posterior pituitary is triggered by changes in both intravascular blood volume and osmolality (*i.e.*, electrolyte-water balance) that activate baroreceptors (McKinley et al., 2004) and osmoreceptors respectively (Bourque et al., 1994). When serum sodium (Na^+) levels rise above 145 mmol/L, the resulting hypernatremia activates the hypothalamic osmoreceptors within the *organum vasculosum lamina terminalis* (OVLT) and the subfornical organ that signal to the supraoptic (SON) and paraventricular nuclei (PVN), and induce AVP secretion by the posterior pituitary into the bloodstream (Kim, 2006, Boone and Deen, 2008). In the kidney, changes in osmolality activate nonselective cation channels which increases the firing rate of action potential and AVP release (Bichet, 2014; van Gastel and Torres, 2017. Conversely, decreased osmolarity promotes AVP retention (Koshimizu et al., 2012). Changes in blood volume, especially when paired to dropped blood pressure (*e.g.*, hemorrhage) activate baroceptors, which also induce AVP secretion (Boone and Dean, 2008).

AVP interacts with transmembrane receptors (R) V1aR, V1bR and V2R expressed by several cell types (Cuzzo et al., 2019; Koshimizu, et al., 2012; Song and Albers, 2018). In the kidney, AVP stimulates water reabsorption to dilute Na^+ in the organism (McKinley et al., 2004; Bourque et al., 1994). Patients suffering from congestive heart failure display high basal levels of AVP, which increases vascular smooth muscle tone (Francis et al., 1984; Goldsmith et al., 1983; Riegger et al., 1982). In the brain, AVP functions as a neuropeptide regulating social behavior (Dumais and Veenema, 2016; Heinrichs et al., 2009; Zink et al., 2011; Goodson and Thompson, 2010).

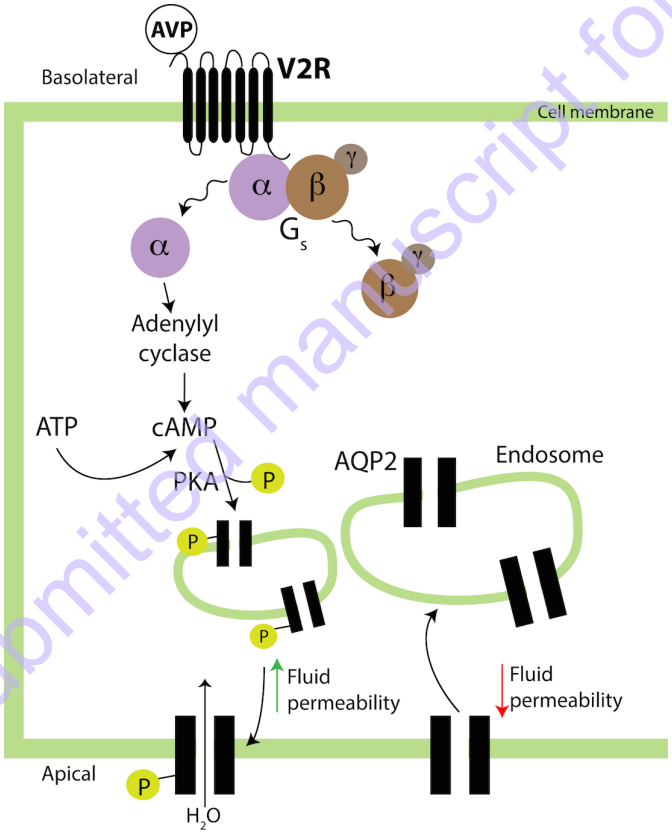
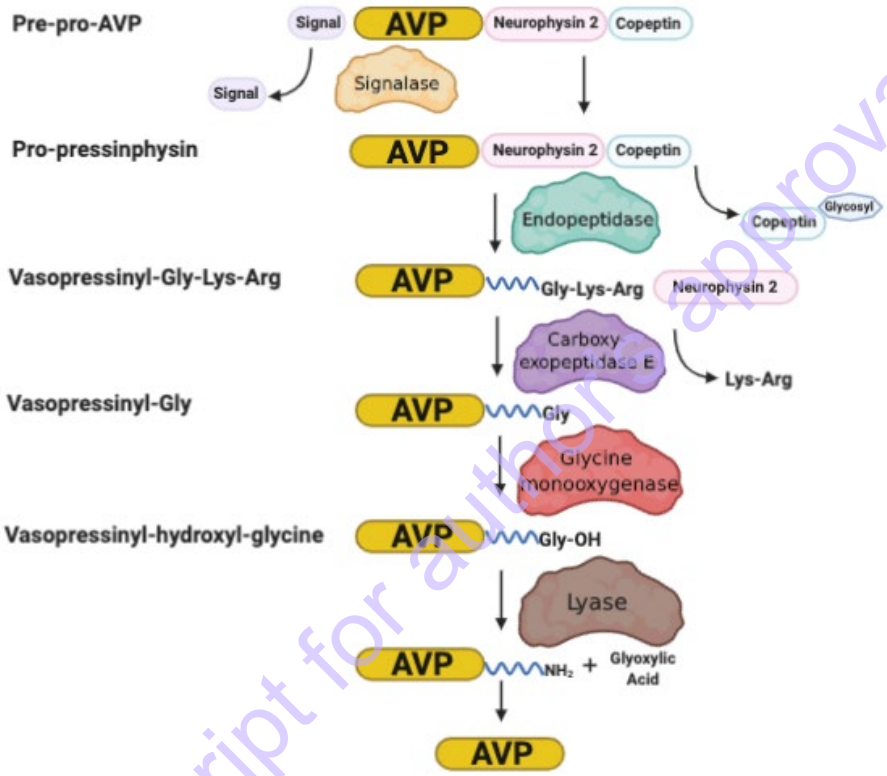


Figure 1. Intracellular response to vasopressin in renal epithelial cells.

AVP binds to the V2R (G-protein coupled receptor) on the basolateral membrane, which activates adenylate-cyclase (AC). Subsequent increase of intracellular cAMP activates protein kinase A (PKA) which phosphorylates the AQP2 water channels stored in vesicular compartments promoting their targeting to the apical membrane. This increases water transport through the membrane (modified after Bichet, 1999).

Figure 2. AVP synthesis.
The hypothalamic neurosecretory neurons synthesize the pre-pro-hormone precursor called AVP-neurophysin-copeptin. The signal peptide of pre-pro-AVP is cleaved by a signalase in the endoplasmic reticulum, to form the pro-hormone called pro-pressinphysin. Copeptin is glycosylated and subsequently cleaved by a Golgi endopeptidase (Iwasaki et al., 1997, Acher et al., 2002; Qureshi et al., 2014). The same endopeptidase also separates the vasopressinyl-Gly-Lys-Arg peptide from neurophysin and the product is enclosed in vesicles. The C-terminal Arg and Lys residues are trimmed by carboxypeptidase E and the newly exposed C-terminal glycine is oxidized by glycine monooxygenase into hydroxyl-glycine. Finally, a lyase converts hydroxyl-glycine is into an amide group which subsequently reacts with glyoxylic acid to yield AVP (Acher et al., 2002).



AVP gene expression. The *AVP* gene is tightly controlled. Firstly, the *AVP* promoter contains a cyclic AMP (cAMP) response element which is bound by the phosphorylated CRE-binding protein in response to increased cAMP (Yoshida, 2008; Kuwahara et al., 2003). The AP1 and AP2 transcription factors promote *AVP* expression, while the glucocorticoid receptor represses *AVP* transcription; note, AP1 and AP2 are also activated in response to stress, infection cytokine and growth factors (Ball, 2017). The *AVP* mRNA is regulated post-transcriptionally through polyadenylation. In the hypothalamic cells of salt-deprived rats, the *AVP* mRNA poly(A) tail length was increased compared to non-salt-deprived rats (Mohr et al., 2001; Zingg et al., 1988). Long polyA tails are likely to increase translation of the cognate protein (Preiss et al., 2000). In mammalian neurons, the poly(A)

binding protein binds the *AVP* mRNA on the "dendritic localization sequence", increases its stability and favors translation (Mohr et al., 2001).

AVP synthesis. AVP is synthesized as a 164 aa long pre-pro-hormone precursor in the cell bodies of the magnocellular neurons of the para- and supra-ventricular nuclei of the hypothalamus (Rotondo et al., 2016) and at lower levels in parvocellular neurons of the PVN (Morales-Medina et al., 2016). Pre-pro-AVP contains an *N*-terminal signal peptide, followed by the AVP and regulatory peptides neurophysin-2 and copeptin open reading frames (ORFs) (Fig. 2) (Waller et al., 1998; Iwasaki et al., 1997). Figure 2 shows how the AVP-containing polyprotein is processed and modified post-translationally (Ball, 2017; Acher et al., 2002, Isawaki et al., 1997).

The pro-hormone is stored in membrane-associated granules and released in response to increased extracellular fluid osmolarity and osmoreceptor activation (Koshimizu et al., 2012). Mature AVP is the nine aa-long peptide Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH2 (reviewed in Rotondo et al., 2016) in which the two cysteines form a disulfide bridge -and thus a ring- and the terminal carboxyl residue is modified post-translationally into a primary amide (NH2) (Yoshida, 2008; Barberis et al., 1998). AVP circulates as a free hormone and is degraded enzymatically in the liver and kidney within 10-30 minutes (Baumann and Dingman 1976).

AVP receptors. The V1aR, V1bR, and V2R are G protein-coupled receptors (GPCRs) with distinct tissue expression (Table 1) which interact with trimeric G proteins, respectively $G_{q/11}$ and G_s (Hoffert et al., 2012).

Table 1. Function and expression of AVP receptors.

TARGET TISSUE	RECEPTOR(S)	FUNCTION(S)	REFERENCE(S)
Kidney – Renal Medulla	V2R	Signal transduction, AQP2 shuttling to	Holmes et al., 2001; Greenberg and

		cell surface and water permeability, *AQP2* mRNA synthesis, increase in intracellular cAMP	Verbalis, 2006; Kato et al., 1995
	V1aR	Vasoconstriction	Park et al., 1997
Vascular Smooth Muscle	V1aR	Vasoconstriction, myocardial hypertrophy, upregulation of *V1aR* mRNA levels, hypertension	Greenberg and Verbalis, 2006; Cottet-Maire et al., 2001; Holmes et al., 2001
Vascular Smooth Muscle	V2R	Vasodilation	Greenberg and Verbalis, 2006
Brain – Anterior Pituitary	V1bR	Adrenocorticotropic hormone (ACTH) secretion, stimulation of endocrine response to stress	Greenberg and Verbalis, 2006; El-Werfali et al., 2015
Brain – abundant expression	V1aR	Regulation of emotional and adaptive behaviors	Johnson and Young, 2017; Carter, 2017
Brain – HPA axis (adrenal cortex)	V1aR	Cortisol synthesis and secretion	Pasquali et al., 1999
Brain – Cerebellum (rats)	V2R	Unknown	Kato et al., 1995
Pancreas	V1bR	Glucagon release, intracellular Ca^{2+} regulation, cell proliferation	Folny et al., 2003; Holmes et al., 2001; Nitschke et al., 1991
Liver – Hepatocytes	V1aR	Glycogenolysis	Greenberg and Verbalis, 2006
Platelets	V1aR	Platelet aggregation	Greenberg and Verbalis, 2006

Myometrium	V1aR	Uterine contraction	Greenberg and Verbalis, 2006
Cervical ganglion (rats)	V1aR	Unknown	Phillips et al., 1990; Zimmermann-Peruzatto et al., 2015
Spleen (rats)	V1aR	Unknown	Zimmermann-Peruzatto et al., 2015
Gonads (rats)	V1aR	Unknown	Phillips et al., 1990; Thibonnier et al., 1996

Kidney. The V1aR receptors are expressed by the vesicular and smooth muscle cells of the renal vessels and mediate the AVP vasopressor effects (Carter, 2017; Wasilewski et al., 2016). V1aR is also expressed in the collecting duct, where upon AVP binding, the G_q protein subunits dissociate and the α_q subunit activates PLCβ which increases diacyl-glycerol (DAG) and inositol triphosphate (IP_3) leading to Ca^{2+} release from the endoplasmic reticulum and activation of the *trp* ion channel which replenishes the Ca^{2+} stores from extra-cellular Ca^{2+} (Fig. 3) (Birnbaumer, 2000). V2R is expressed in the nephron, in the basolateral membrane of epithelial cells of the distal convoluted tubule and collecting duct and mediates the AVP antidiuretic action (Machida et al., 2007; van Gastel and Torres, 2017; Sebti et al., 2015). In the collecting duct cells, AVP binding to V2R dissociates the receptor from the G_s protein subunits, which activates a signal transduction cascade producing cAMP and releasing Ca^{2+} from ryanodine-sensitive stores (Fig. 3) (Hoffert et al., 2005). Such cascade leads to protein kinase A (PKA)-dependent phosphorylation of aquaporin 2 (AQP2) (Robben et al., 2004). AQP2 is a water channel/transporter stored in intracellular vesicles (Agarwal and Gupta, 2008). Upon AVP signaling, such vesicles are shuttled to and fused with the apical membrane, which enhances water reabsorption (Fig. 1) (Olesen and Fenton, 2017;

Park and Kwon, 2015). AVP can be translocated to the cytoplasm and degraded by the proteasome, which dampens signaling and promotes water excretion and formation of diluted urine (Friberg et al., 2004; Gubbi et al., 2019; Shi et al., 2017). Fluid levels and water homeostasis relate directly to intravascular blood volume and osmolality.

Heart. Myocytes of the cardiac vascular smooth muscle express the V1aR (Singh et al., 2014; Wasilewski et al., 2016). While AVP was shown to protect the heart from myocardial injuries (Nazari et al., 2011; Zhu, 2014), both high circulating AVP and V1aR over expression have been associated with heart failure, indicating that the strength of V1aR signaling appears important (Wasilewski et al., 2016).

Brain. In the brain, V1aR, V1bR and V2R mediate the adaptive behavioral responses to AVP. Typically, the brain displays species-specific AVP receptor expression patterns, with V1aR and V1bR most commonly found in the lateral cortex (Johnson and Young, 2017; Carter, 2017). The human corticotrophic cells of the anterior pituitary express high levels of V1bR, which promotes production of the adrenocorticotropic hormone (ACTH) that stimulates the adrenals to synthesize and secrete cortisol, androgens, and aldosterone (Jasnic et al., 2013; van Gastel and Torres, 2017; Verbalis et al., 2009). Under stress conditions, V1bR contributes to activate the hypothalamo-pituitary-adrenal axis (Tanoue et al., 2004). Interference of the AVP-V1bR interaction by means of the V1bR antagonist Nelivaptan, reduced ACTH secretion by approximately 15% (Jasnic et al., 2013). V1bR is found in the CA2 region of the hippocampus and the anterior region of the amygdala of the brain, which implicates AVP in social behavior and development (Stevenson & Caldwell, 2012). RT-PCR showed that the *V2R* mRNA is expressed both in the cerebrum and in the hippocampus of newborn rats (Kato et al., 1995). V2R

mRNA levels appeared constant in the former and decreased with age in the latter (Kato et al., 1995). V2R function in the brain is currently unclear (Dumais and Veenema, 2016).

Other tissues. In the pancreas of rodents and humans, AVP binding to V1bR augments insulin release (Folny et al., 2003, Mohan et al., 2019). Using the selective non-peptide V1bR antagonist SSR-149415 abolished Ca^{2+} release, glucagon secretion, and cell proliferation of a glucagon-secreting α-pancreatic cell line (Folny et al., 2003). Similarly, murine, rodent, and human pancreatic β-cell isolates treated with the V1bR antagonist Nelivaptan abolished insulin release (Mohan et al., 2019). As seen by RT-PCR, the *V1bR* mRNA is also expressed in the adrenals and small intestine, albeit how these cells respond to AVP remains to be defined (Folny et al., 2003).

Regulation of AVP receptor expression. Expression of the AVP receptors responds to several tissue-specific signals. For instance, in the collecting duct metabolic acidosis increases V1aR (Tashima et al., 2001) and decreases V2R expression (Machida et al., 2007). Conversely, dehydration upregulates V2R expression (Machida et al., 2007). V2R engagement by AVP stimulates ubiquitin-dependent degradation, which is faster than steady-state turnover (Martin et al., 2003). V1bR responds to stress signals both transcriptionally and translationally (Kashiwazaki et al., 2015; Goncharova et al., 2013). Furthermore, V2R expression levels are sex-dependent, which may have a bearing for AVP-V2R-linked pathologies (*e.g.*, PKD, below, Liu et al., 2011).

Receptor desensitization. Alike other GPCRs, the signal cascade from AVP receptor activation also results in receptor desensitization which interrupts downstream signaling (Birnbaumer et al., 1992, Nathanson et al., 1994, Ancellin et al., 1998). This occurs through GPCR phosphorylation by GPCR kinases, arrestin binding, G protein uncoupling and receptor

internalization. V1aR is rapidly recycled to the cell surface. In contrast, V2R is sequestered in perinuclear recycling compartments (Innamorati et al., 1998a, 1998b, 2001). Because AVP receptors can be phosphorylated by other kinases as well, heterologous desensitization can occur in response to different signals (*e.g.*, angiotensin II) (Ancellin et al., 1999).

Polycystic kidney disease and AVP

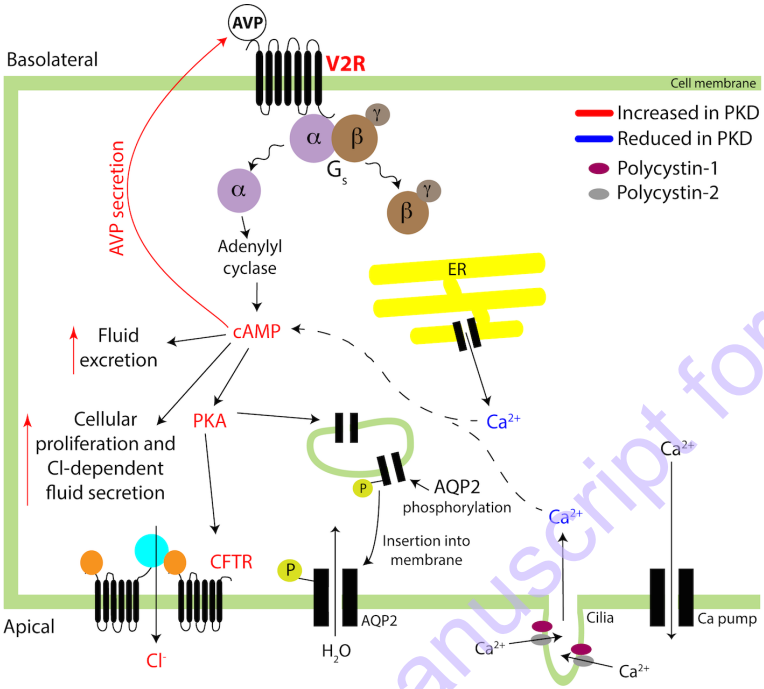


Figure 3. V2R and AVP signaling in normal and ADPKD renal cells. In normal cells, AVP binding to V2R promotes dissociation of the trimeric Gs into its α and βγ subunits. The α subunit triggers adenylyl cyclase (AC)-mediated cAMP synthesis, which activates PKA and phosphorylates AQP2. Phospho-AQP2 is shuttled to the apical cell membrane. In ADPKD, reduced Ca^{2+} release from the ER and impaired Ca^{2+} import from polycystin 2 at the primary cilia elevate intracellular cAMP, which in turn promotes fluid excretion. Dehydration from excessive water excretion triggers release of AVP from the pituitary. Furthermore, higher than normal intracellular cAMP in ADPKD boosts Cl^- transport via the CFTR channel that contributes to cystic cell proliferation and Cl^--dependent fluid secretion.

In PKD, cystic degeneration of the kidneys progressively affects their function, disrupting water balance. Autosomal dominant PKD (ADPKD) is a hereditary renal disease affecting about 12.5 million people worldwide (Bergmann et al., 2018). The majority of mutations found in ADPKD patients map to the *PKD1* and *PKD2* genes (Harris and Torres, 2009; Bergmann et al., 2018). In autosomal-recessive PKD (ARPKD), a less common form, mutations affect the *PKHD1* gene (Bergmann et al., 2004; Wang et al., 2008; Bergmann et al., 2018). *PKD1*, *PKD2*, and *PKHD1* all encode transmembrane proteins, namely polycystin 1, polycystin 2 and fibrocystin (Wang et al., 2008).

Polycystin 1 is considered an orphan, atypical GPCR. Polycystin 2 a Ca^{2+} permeable non-selective cation channel with homology to the transient receptor potential (TRP) superfamily (Hughes et al., 1995, Hanaoka et al., 2000, Bai et al., 2008). Fibrocystin, also called polyductin is also a transmembrane protein (Onuchi et al. 2002, Ward et al., 2002, Xiong et al., 2002). Polycystin 1, and 2 and fibrocystin interact and can be found at the cilium, considered critical in PKD (Hanaoka et al., 2000, Bai et al., 2008). The precise mechanisms of cyst formation and growth are unknown however, multiple changes are known to occur at the molecular, cellular, and physiological level that affect tubular homeostasis and function. Neoplastic-like cystic growth and tubular epithelial cell apoptosis characterize PKD (Bergmann et al., 2018, Torres et al., 2017). Early PKD stages are characterized by abnormally high fluid excretion, which causes dehydration and activates compensatory AVP release (Bergmann et al., 2018; Torres et al., 2017; Seeger-Nukpezah et al., 2015). While in ADPKD congenital cysts are only present in 1-3% of nephrons, continued cystic growth and new cysts eventually deform the surrounding tissues mechanically, impair surrounding nephrons and increase cell death. At advanced PKD stages, kidneys can quadruple their size and reach the size of a football (Bergmann et al., 2018). These events eventually overwhelm renal compensation, causing kidney failure in half of the ADPKD patients (Bergmann et al., 2018, Torres et al. 2017). *AVP* loss of function in the PCK rat ($AVP^{-/-}$) results in defects reminiscent of autosomal recessive polycystic kidney disease (ARPKD) with increased renal cAMP and ERK phosphorylation (Wang et al., 2008).

Molecularly, AVP, V2R and cAMP signaling are all altered in PKD (Nagao et al., 2006; Wang et al., 2008; Reif et al., 2011; Rinschen et al., 2014; Wu and Yu, 2016). *PKD1* and *PKD2* mutations reduce Ca^{2+} signaling in the primary cilia and endoplasmic reticulum (ER) of the epithelial cells of the renal tubule, which in turn increases intracellular cAMP levels and fluid excretion (Chebib

et al., 2015) (Figure 3). De-regulated higher excretion causes dehydration and the compensatory release of AVP to stem water loss (Chebib et al., 2015; Devuyst and Torres, 2013; Torres and Harris, 2006). Concomitant up-regulation of V2R expression in PKD kidneys further increases intracellular cAMP levels, which in turn increases protein kinase A activity and Cl^- secretion *via* the cystic fibrosis transmembrane conductance regulator (CFTR), fueling cystic cellular proliferation and Cl-dependent fluid secretion (Pinto et al., 2012; Torres and Harris, 2006). ARPKD cells also show AVP/V2R upregulation, cAMP-activated cellular proliferation and reduced intracellular Ca^{2+} (Belibi et al., 2004; Nagao et al., 2006; Gradilone et al., 2010). Finally, in PKD, V2R may redistribute apically in the tubular cells (Rinschen et al. 2014). Therapeutic targeting of the AVP system with V2 antagonists appears to be moderately effective in the short term and is discussed below.

Biological sex and PKD. In human and murine species, higher V2R mRNA expression in females vs. males (Lui et al., 2011; reviewed in Juul et al., 2014) may imply existence of phenotypic, clinical and/or pharmacological differences in men and women. The relationship between sex and PKD appears complicated and is incompletely understood. Notably, while men appear more sensitive than women to several kidney diseases, in ADPKD such difference appeared to be reduced, implicating that women may instead have faster disease progression (Ishikawa et al., 2000). More than 80% of ADPKD and severe polycystic liver disease patients are females, indicating that hormonal regulation may play an integral role in disease severity (Bergmann et al., 2018). However, in a recent study, female patients displayed slower cystic progression than males, adding to a recent proposal that men may be more severely compromised by ADPKD than women, despite the latter being more affected numerically (Bae

et al., 2019). In ARPKD, males and females are equally affected (Bergmann et al., 2018). Future studies will be needed to clarify this potentially important aspect of the PKD pathophysiology.

AVP and Heart Failure.

As of the year 2015, cardiovascular disease had caused the death of 17.3 million people worldwide to which HF has been one of the leading causes (Mozaffarian et al., 2015). The decline in the heart's ability to effectively operate as a fluid pump and maintain proper systemic circulation, HF translates clinically into decreased cardiac output and stroke volume, regardless of total circulatory blood volume (Ishikawa, 2014). Chronic HF patients exhibit two to three times higher serum concentrations of AVP (2.5 - 6.4 pM) compared to healthy individuals (< 1.6 pM). Higher AVP levels are associated with later stages of HF and suggest that AVP may contribute to disease progression (Chen et al., 2015, 2016). AVP synthesis within the SON and the PVN is coordinated with the afferent signaling pathways from baroreceptors localized within the aorta, the carotids, the cardiac atria and left ventricle (Stewart et al., 1988; Szmydynger-Chodobska et al., 2011). Essentially, in HF the decrease in circulating blood volume results in reduced baropressor sensitivity, leading to non-osmotic AVP release (Ishikawa, 2014). As a vasoconstrictor, AVP increases blood flow. Specific to HF, systemic release of AVP increases peripheral vascular resistance and compensates for reduced cardiac output and stroke volume in the short-term (Stewart et al., 1988; Hiroyama et al., 2007). In chronic HF, however, AVP hyperstimulation eventually impairs the heart mechanical function, promotes extensive cardiac remodeling and causes fluid imbalances synergistically exacerbating cardiac dysfunction (Yang et al., 2003; Chen et al., 2015).

AVP-V1aR signaling and cardiac contractility. In HF, AVP secretion appears to correlate with V1aR upregulation. The left ventricular myocardium of HF patients with elevated plasma AVP levels displayed higher *V1aR* mRNA level (Zhu et al., 2014). Similarly, HF induced by left coronary ligation in the *Ntac: SD* $^{+/+}$ rat also featured increased *V1aR* mRNA expression within the left ventricle (Czarzasta et al., 2019). Probing of the AVP-V1aR signal transduction pathway revealed that controlled overexpression of the human V1aR in mice cardiac myocytes, ($V1a^{tTa/+}$) augmented AVP-V1aR signaling and promoted Gαq protein recruitment to the plasma membrane which increased D-myo-inositol 1,4,5 trisphosphate signaling and Ca^{2+} mobilization (Fig. 4A). V1aR overexpression lead to extensive myocardial contraction, hypertrophy, vasoconstriction and reduced cardiac contractility (Li et al., 2011; Goldsmith et al., 2005). Persistent AVP-V1aR signaling can also disrupt β-adrenergic receptor activation and signaling (Fig.4B) (Tilley et al., 2014). Hence, physiological coupling of elevated AVP secretion and V1aR density appears a main inducer of changes in cardiac contractility and morphology which accelerate cardiac dysfunction.

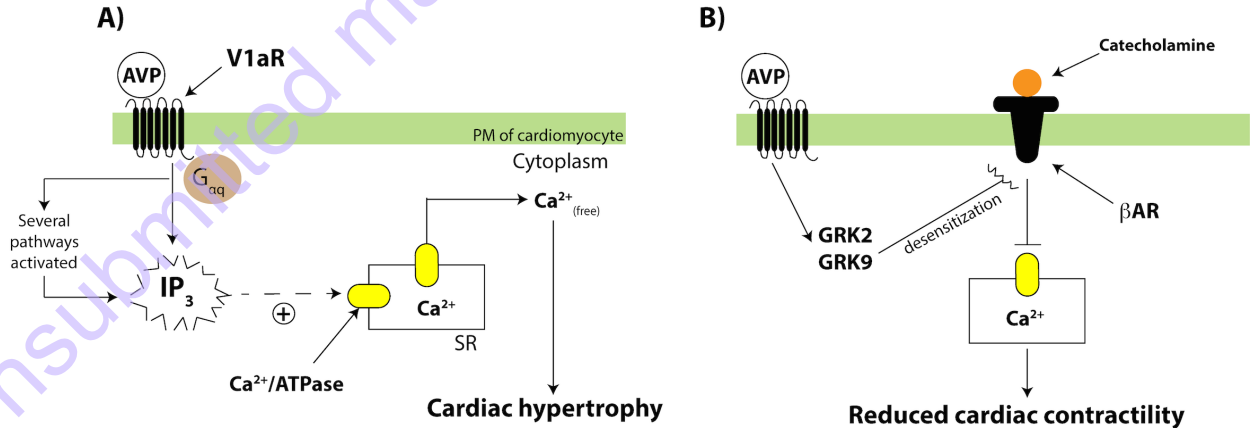


Figure 4. AVP-V1aR signaling contributes to weaker myocardial contractions and cardiac remodeling in HF. **(A)** AVP binding to V1aR during HF activates $G_{\alpha q}$ protein-mediated signaling, which amplifies IP_3 signaling and triggers Ca^{2+} release from the sarcoplasmic reticulum (SR). Prolonged Ca^{2+} mobilization leads to myocardial hypertrophy. **(B)** $G_{\alpha q}$-independent signaling promotes GRK recruitment to the plasma membrane (PM) decreasing

catecholamine-β adrenergic receptor (βAR) activation and Ca^{2+} mobilization. When prolonged, this condition impairs myocardial contractions.

AVP-V1aR signaling and cardiac remodeling. Cardiac fibroblasts (CFs) are non-contractile cells amounting up to 60% of the heart. CFs are essential to maintain the structural integrity of the heart's extracellular matrix (ECM) (Yang et al., 2003). Activation of V1aR signaling was found to promote CF proliferation and function (Chen et al., 2015; Yang et al., 2003). In CFs, AVP-V1aR dependent signaling appeared to recruit GRK2 and β-arrestin1/2 and increase expression of matrix metalloproteinases MMP2 and MMP9, functioning in ECM degradation and tissue remodeling (Chen et al., 2016; Chen et al., 2015). Moreover, AVP-V1aR signaling induces phosphorylation of mitogen-activated ERK1/2 kinase that binds GRK2 and β-arrestin1/2 towards stimulation of CF proliferation (Chen et al., 2016; Hiroyama et al., 2007) and increased expression of connective tissue growth factor and endothelin-1 that respectively promote collagen synthesis and inhibit MMP1, a process leading to extensive ECM deposition (Chen et al., 2015). The vasopressor function of AVP and endothelin-1 combined contributes to increase peripheral vascular resistance and cardiac afterload, which -when prolonged- causes adaptive myocardial hypertrophy to try maintaining efficient cardiac output to the periphery (Ishikawa, 2014; Stewart et al., 1988).

AVP-V2R signaling for fluid volume retention and cardiac function. AQP2 upregulation in a coronary-ligated rat model of congestive HF, was shown to lead to greater infarction of the left ventricular free wall and further impair cardiac performance (Xu et al., 1997; Xu et al., 1991). In light of continued AQP2 translocation to the apical membrane, increased systemic fluid retention and fluid saturation/ hypervolemic state results in higher ventricular filling (cardiac preload). If sustained, this condition can stress the diastolic wall, promote MMP activation, and cardiac hypertrophy (Chen et al., 2015). Altogether, chronic AVP secretion coupled with activation of

V1aR and V2R signaling contribute to a vicious cycle of extensive myocardial remodeling and inefficient contractile events, usually escalating into fatal HF (Brønd et al., 2013; Xu et al., 1991).

AVP and Brain Function

AVP functions as a neurohormone which affects memory and attention (Neumann et al. 2008; Carter, 2014), increases neural transmission in the amygdala (Huber et al., 2005; Meyer-Linderberg et al., 2011) and participates to the neuroendocrine stress response (Axelrod and Reisine, 1984). AVP also modulates social behaviour in several fish, amphibians, vertebrates and mammalian species with sexual dimorphic displays (reviewed in Dumais and Veenema, 2015; Goodson and Bass, 2001).

The AVP-synthesizing magnocellular neurons of the hypothalamic PVN project to the posterior pituitary, whence AVP release into the circulatory system regulates water retention (Buijs et al., 1983; Leng et al., 1999; Antoni, 1993; Landgraf and Neumann, 2004). In contrast, the parvocellular neurons of the PVN project to the median hypothalamic eminence whence AVP release triggers, instead, the secretion of ACTH and anterior pituitary hormones (Armstrong, 2014; Antoni, 1993). Other AVP-synthesizing neurons can be found in the medial amygdala, the bed nucleus of the stria terminalis (BNST) and the suprachiasmatic nucleus (SCN) which all project centrally to distinct areas such as the brain preoptic and olfactory areas, and both hypothalamic and extra-hypothalamic regions (Sofroniew, 1983; Albers, 2015; Holmes et al., 2016, Wacker and Ludwig, 2018). These neurons are thought to be the source of neural AVP because the blood brain barrier is practically impermeable to plasmatic AVP (Ludwig and Leng 2006; Ludwig and Stern, 2015; Meyer-Lindenberg et al. 2011, Motoki et al., 2016). Seven of the nine amino acids in AVP are identical to oxytocin (OT), another nonapeptide and neurohormone whose gene partially overlaps with the *AVP* gene (Sausville et al., 1985; Acher et al., 1995).

AVP and OT can function antagonistically, and differentially to sex and several context-dependent variables. AVP acts as an anxiogenic and OT as anxiolytic and pro-social (Legros, 2001; Carter et al., 2008; Heinrichs et al., 2009; Neumann and Landgraf, 2012; Stoop, 2012, Motoki et al., 2016). The distinct expression and distribution of AVP, OT, their receptors, and the reach of their neuronal projections further intertwines these systems (reviewed in Meyer-Linderberg et al., 2011, Johnson and Young, 2017). Recently, it was found that the similarity between OT and AVP results in their binding to each other's receptors and substantial crosstalk in vivo, especially at high AVP and OT concentrations (Song and Albers, 2018). Integrative functional models of AVP and OT are being developed to reconcile a large body of observations made in disparate experimental set ups and behavioural paradigms, primary focus on OT, focus on male individuals, and the inherent complexity of the neural circuitry regulating social behaviours. Here, we will examine primarily the AVP functions, referring readers interested in the biology of OT to comprehensive reviews (Carter, 2014; Meyer-Linderberg et al., 2011).

AVP and animal behaviour. AVP and its non-arginine relatives (VP) preside to sociality in several rodents (rats, mice, hamsters, voles, jerboas) as well as birds and fish through largely conserved neural networks including the amygdala, BNST, lateral septum, the medial preoptic area, anterior hypothalamus and the periaqueductal grey (De Vries and Panzica, 2006; Donaldson and Young, 2009; Engelmann et al., 2000; Ferris, 1992; Gilligan et al., 2003; Goodson and Bass, 2001; Goodson, 2005; Johnson and Young, 2017; Moore and Lowry, 1998; Newman, 1999; Storm and Tecott, 2005). Specific behavioural responses appear both species-specific and may differ among conspecifics, likely due to differential distribution and expression of neural AVP receptors, as well as the influence of gonadal hormones (Donaldson and Young, 2009; Goodson and Bass, 2001; Albers, 2015). Gene knock-out of *AVP* or its receptors, and

pharmacological targeting of AVP receptors in vivo have been used in combination with behavioral tests such as the forced swim test (assesses the animal's overwhelm from prolonged drowning threat) and elevated plus maze (measures anxiety; Morales- Stevenson and Caldwell, 2012; Medina et al., 2016; reviewed in Caldwell, 2017) to probe AVP neural functions. The AVP response appeared to be affected by the conditions of the subjects, including their social experience and hormonal status as well as by the connections of neurons within the hypothalamus and between the hypothalamus and other areas. Both V1aR and V1bR are widespread in the brain and play distinct functions (Table 1) (Griebel and Holsboer, 2012; Wacker and Ludwig, 2018; Wu et al., 2015).

V1bR. V1bR engagement by AVP in the anterior pituitary and adrenal medulla promotes release of key stress hormone ACTH (Roper et al., 2011; Koshimizu et al., 2012; Barsegyan et al., 2015). This is additive to the V1aR-mediated synthesis and release of cortisol in the adrenals (Pasquali et al, 1999). In the stress of the forced swim test and elevated plus maze, male *V1bR*$^{-/-}$ mice displayed reduced resting levels and impaired ACTH release, compared to controls (Tanoue et al., 2004; Koshimizu et al., 2012). These responses appeared stressor specific, implicating nuanced underlying mechanisms. Studies of the *V1bR*$^{-/-}$ mice have revealed social deficits (Wersinger et al., 2002, 2004) and that V1bR may mediate aggression (Wersinger et al., 2007; Bosch, 2013).

V1aR. V1aR may regulate individual recognition, pair-bonding, sexual behaviour, social memory, and aspects of parental care (e.g., maternal aggressive behaviour, anxiety, depression) (Goodson and Bass, 2001; Ferguson et al., 2002; Bales et al., 2004; Bielsky et al., 2004; Albers, 2012; Koshimizu et al., 2012). Intracerebral AVP microinjection and V1aR overexpression in the lateral septum both improved social recognition; conversely, both *V1aR* knockout and

administration of V1aR antagonists severely impaired sociality (Bielsky et al., 2004; Stemmelin et al., 2005; Frazier et al., 2006; Clipperton-Allen et al., 2012).

Recognition. In rodents, species-specific recognition is largely based on olfactory cues, which appear reflected in species-specific distribution and type of AVP-immunoreactive (ir) nerve fibers (Wacker et al., 2011). Pharmacological or genetic V1aR impairment also prevented short-term social recognition in rats, possibly through AVP-induced changes in olfactory processing (Pineda et al., 2017; Tobin et al., 2010; Wacker and Ludwig, 2018).

Aggression. AVPir neurons in the medial amygdala and BNST that project to the lateral septum and AVPir projections to the anterior hypothalamus are implicated in aggression in rodents (De Vries and Buijs 1983; Goodson, 1998; Goodson and Adkins-Regan, 1999; Koolhaas et al., 1998, Goodson and Bass, 2001). While this theme is conserved in several species, anatomical specializations are thought to mediate species-specific behaviours. For example, VP infusion into the septum inhibited aggression against intruders in the field sparrow, a territorial species (Goodson, 1998), whereas it increased aggression in the colonial zebra finch (Goodson and Adkins-Regan, 1999). Note, the septum is involved in social cognition, stress response and anxiety.

Parental care. In rodents, parental care (e.g., pup licking and grooming) is necessary for both early development and the adoption of similar nurturing behaviour as adults. Genetic and pharmacologic evidence indicated that in both sexes, pup grooming and maternal post-partum aggression are mediated by the AVP/VP network and neuronal connection between the hippocampus and both the amygdala and the basal forebrain (reviewed in Zimmermann-Peruzzatto et al., 2015). Engaging in parental behaviour increased expression of both AVP and

its receptors, which consolidates gene expression and behaviour (Pedersen et al., 1982, 1985, 1994; Wang et al., 1994; Albers 2012).

Sexual behaviours. The effects of AVP/VP on sexual behaviour have been reviewed elsewhere (Argiolas and Melis, 2013).

Differential sex response to AVP. In most species (except hyenas and rats) estrogen and androgens seem to increase AVP levels (Albers, 2015). In rats, testosterone was found to modulate AVP/VP receptor expression and their localization in the brain, as well as the number of AVP producing cells (De Vries and Al Shamma, 1990; Urban et al., 1991; De Vries et al., 1994; Delville et al., 1996). Conversely, castration reduced AVP expression in a subpopulation of cell bodies within the BNST of amphibians, birds, and mammals (Albers, 2015; Lebow and Chen, 2016). Note, the BNST is a sexually dimorphic centre that integrates limbic information and valence monitoring and has been implicated in several psychiatric disorders. Other observations corroborate the importance of AVP signaling strength. Compared to females, male rats showed denser AVP-immunoreactive nerve fibers in the lateral septum and lateral habenular (De Vries et al., 1981; Fink et al., 1991, De Vries et al., 2009) that may impact a subset of AVP/VP responses (Garcia-Villalon et al., 1996; reviewed in Dumais and Veenema, 2016). In naked mole rats, breeding dominant males and females were found to contain more AVP-positive neurons than subordinate animals (Albers, 2015; Rosen et al., 2007). In mandarin voles dominant and subordinate females displayed different distribution of AVP-containing neurons in the PVN and SON (Qiao et al., 2014).

Changes in AVP-signaling. Because the AVP system is highly adaptive, it responds to conditions and environment. For example, maternal stress during late pregnancy reduced *V1aR* neural expression and impaired pup sociality (Grundwald et al., 2016). Seasonal breeding

(Goodson et al., 2012; Hermes et al., 1990) and fatherhood (Bamshad et al., 1993; Lambert et al., 2011; Perea-Rodriguez et al., 2015) also modulate AVP signaling.

Circadian response. V1aR signaling affects circadian rhythmicity (Tsuji et al. 2017; Li et al., 2009). In mice, circadian non endocrine regulation elicited by SCN neurons triggers AVP release, OVLT activation and increases thirst before sleep to prevent dehydration during the night hours (Gizowski et al., 2016, 2018).

AVP and human behaviour. The AVP (and OT) systems underscore the neural underpinning of human social cognition, with acute effects on context-specific behavioural responses and long-term behavioural regulation (e.g., anxiety, reward, Crockford et al., 2014, Feldman et al., 2016). OT is pro-social (Preckel et al., 2014; Scheele et al., 2012; King et al., 2016; Chen et al., 2016; Petrovic et al., 2008). On context, AVP can either promote pair bonding and cooperation or threat reaction and anxiety (Brunnlieb et al., 2014; Rilling et al., 2014). Underscoring the important role of AVP in social behaviours, patients with certain personality disorders displayed elevated AVP in the cerebrospinal fluid and often systemically (Coccaro et al., 1998; Landgraf and Neumann, 2004). Autism spectrum disorder (ASD), Williams syndrome, schizophrenia, depression, social anxiety, and attachment disorders all respond to AVP receptor blockade or are thought to be linked to the AVP and OT systems (Bielsky et al., 2005; Bookheimer et al., 2008; Dai et al. 2012; Dalton et al., 2007; 2005; Guastella and Hickie 2016; Hadjikhani et al., 2007; Meyer-Linderberg et al., 2009, 2011; Rubin et al., 2013; Zhang et al., 2017; Zink et al., 2009). Reduced AVP levels were found in patients with schizophrenia and bipolar disease, with low AVP levels possibly predisposing to psychoses, regardless of OT (Rubin et al., 2013, 2014). Social stress is known to increase odds to develop psychiatric disorders (Selten et al., 2005) and AVP signaling appears linked to the limbic system at least via

the function of the serotonin transporter SLC6A4 (Bachner-Melman et al., 2005; Grace et al., 2018; Lefevre et al., 2018; Meyer-Linderberg et al., 2006; Pezawas et al., 2005). Using a rat model of early life trauma and patient observations, increased AVP signaling was shown to be causative in borderline personality disorder, regardless of sex or stressor (Brydges et al., 2019). Underscoring the complexity of the AVP neural network, in other experimental settings, the AVP system was found to respond to gonadal steroids, and individual responses to specific contexts appear influenced both by genetic background (more below) and life history. One possible factor contributing to such diversity may be that sensitivity to OT signaling (and its AVP-balancing functions) seems to be established early in life (Meinlschmidt and Heim 2007). The AVP-responsive amygdala, cingulate gyrus, and hypothalamus all affect social behaviour (Goossel et al., 2009; Insel and Young, 2001; Meyer and Linderberg et al 2009). Recently, AVPir projections have been demonstrated in the human male agranular insula (Rogers et al., 2018) a region in the brain neocortex presiding to sensory processing, high-level cognition, and affection (Uddin et al., 2017). Thus, AVP appears to be central to neural developmental pathways. Polymorphic microsatellites in the regulatory regions upstream of the *V1aR* gene may influence expression levels and amygdala activation (Kim et al., 2002; Mabry et al., 2011; Hammock and Young 2005; Wassinck et al., 2004; Thibonnier et al., 2000). Some *V1aR* gene polymorphisms associate with behavioural traits, including novelty seeking, sexual behaviours, musicality, and dance (Bachner-Melman et al., 2005; Guastella et al., 2011; Meyer-Lindenberg et al., 2009; Walum et al., 2008; reviewed in Meyer-Lindenberg et al., 2011). Single nucleotide polymorphisms and simple sequence repeats analyses in the *V1aR* genes revealed associations with schizophrenia and autism, social cognition (reviewed in Zink et al., 2011), and social behaviours including aggression, altruism, depression empathy (Aspé-Sanchez et al., 2016;

Rubin et al., 2014; Ben-Efraim et al., 2013). *AVP* gene polymorphisms reducing expression were linked to schizophrenia (Jobst et al., 2014). Consistent with a causal relationship, AVP administration appeared to lessen negative symptoms of some schizophrenic patients (Rubin et al., 2013).

Intra-nasal administration is used to study the effects of AVP in human subjects in combination with functional MRI (fMRI) to reveal the brain areas responding to AVP by detecting changes in deoxyhemoglobin concentration (Born et al., 2002; Glover, 2011; Heinrichs et al., 2009; Wacker and Ludwig, 2019). As in animals, intranasal AVP administration may increase endocrine stress signaling by augmenting activity of the amygdala (Huber et al., 2005) and promoting ACTH secretion (Axelrod et al., 1984) and may favor negative responses in ambiguous social situations (Thompson et al., 2004). While AVP reduced friendliness in males, it increased it in females (Thompson et al., 2006). Brain fMRI in a Prisoner's Dilemma Game revealed that, upon intranasal administration of 20 IU of AVP and compared to placebo, AVP increased the activity of the BNST and reciprocated cooperation in men (Rilling et al., 2012, 2014). The BNST responds to AVP and OT and interfaces with brain areas regulating affiliation, parental care, sexual behaviours, communication, and aggression (Goodson and Kingsbury, 2013; Newman, 1999). In contrast, in women, AVP activated the left caudate nucleus and left amygdala and instead increased cooperation following partner defection (Rilling et al., 2012). In men, perceived unreciprocated cooperation appeared to reduce activation of the right amygdala, which is responsible for processing negative emotions, and anterior insula (Chen et al., 2017). Interestingly, these effects may be influenced by personality traits. Upon AVP administration, male participants who scored high on a neuroticism test also showed higher activity in the anterior cingulate cortex, medial prefrontal cortex and lateral temporal lobe in response to

unreciprocated cooperation (Feng et al., 2015). Note, such regions normally mediate higher functions including emotion, decision-making, and language. Conversely, cooperation increased activation of the right insula, which is linked to body awareness (Feng et al., 2015). AVP increased male cooperation in choosing financially riskier, yet mutually rewarding choices through decreased activity of the left dorsolateral prefrontal cortex (which modulates cognitive flexibility during risky cooperation) (Brunnlieb et al., 2016). AVP may alter male emotional processing. Seeing facial display of fear or anger normally changes activity in the subgenual cingulate of the medial prefrontal cortex, which is part of the limbic system presiding to emotional processing. However, men treated with 40 IU of intranasal AVP, neither recognized the difference nor displayed differential brain activity, and showed decreased functional connectivity between the subgenual and supragenual cingulate (Zink et al., 2010). Thus, the human AVP response shows shared mammalian and avian traits, as well as critical species- and individual- specific responses due to individual genetics, life history, and context, which must all be considered to define how AVP integrates sensory processing and behaviour.

Pharmacological modulation of the AVP pathways.

The human AVP pathway is targeted pharmacologically using AVP receptor agonists and antagonists.

Vasopressin Receptor Antagonists. Also known as vaptans, AVP receptor antagonists are a group of nonpeptide drugs administered orally or intravenously that bind specifically to renal and cardiac V2R and increase aquaresis i.e., the excretion of solute-free water (Wu et al., 2017; Narayen and Mandel, 2012). Tolvaptan, Lixivaptan, and Satavaptan each have distinct chemical structures that uniquely influence affinity and biological activity (Table 2). Upon

receptor interaction, the vaptans prevent translocation of the aquaporin water channel to the apical membrane of the renal collecting duct (Rangarajan et al., 2014; Mise et al., 2019; Farah et al., 2010). Expanding the biological targets, conivaptan exhibits high affinity for both the V1aR and V2R. Upon binding to localized V1aRs, conivaptan can limit myocardial hypertrophy (Ali et al., 2007; Finley et al., 2008). Balovaptan selectively binds to V1aRs within neural tissue and has potential for treating human behavioral disorders (Bolognani et al., 2019; Schnider et al., 2020).

V2R antagonism in heart disease therapy. Because of their aquaretic effects, the vaptans are used in hyponatremic patients with congestive HF with simultaneous hypervolemic hyponatremia and edema (Narayen and Mandel, 2012). Administration of 30-45 mg of tolvaptan can reduce edema, body weight, and serum sodium level without compromising blood pressure and renal function (Wu et al., 2017).

V2R antagonism in PKD therapy. Based on the strong AVP deregulation observed in animal ADPKD and ARPKD models, strategic reduction of AVP-mediated signaling and intracellular cAMP using V2R antagonists was found to decrease both cystic fluid secretion and cyst size in murine PKD models (Torres et al., 2004; Wang et al., 2008; Meijer et al., 2011) and PKD patients (Torres et al., 2017; Torres et al., 2012; Reif et al., 2011). The V2R antagonist and benzazepine derivative OPC31260 (Yamamura et al., 1992) reduced cystogenesis and inhibited new cyst formation in several PKD rodent models (Wang et al., 2008; Torres et al., 2004) as well as nephronophthisis (Wang et al., 2005). OPC31260 can displace AVP from V2R and V1aR (IC50 respectively 1.4 x 10^{-8} M and 1.2 x 10^{-6} M) (Yamamura et al., 1992). In the ADPKD *Pkd*$^{-/tm1Som}$ mice, OPC31260 lowered renal cAMP, AQP2 and V2R expression (all higher than normal in this model and ADPKD patients) and reduced kidney weight to wild-type levels (Torres et al., 2004). OPC31260 also improved the PCK *AVP*$^{-/-}$ ARPKD rat, reducing cAMP, kidney-specific

ERK phosphorylation, and overall improving water reabsorption, reducing kidney cysts and weight compared to controls (Wang et al., 2008). More recently, tolvaptan, which was already approved for the treatment of heart disease in many countries, was tested in clinical trials for the treatment of PKD patients.

Tolvaptan in PKD. Tolvaptan inhibited ADPKD-type cystic cell proliferation fueled by the AVP-induced activation of B-Raf/MEK/ERk pathway and decreased another contributor to cystic growth i.e., the AVP-stimulated Cl^- secretion cyst (Reif et al., 2011). Following promising results from the PKD experimental models, tolvaptan, was clinically tested as possible ADPKD therapy. The phase 3 TEMPO clinical trial included 1445 patients, between the ages of 18 and 50, who had a kidney volume of at least 750 ml (i.e., 50% increase compared to healthy) and a creatinine clearance of at least 60 ml/min (moderate decline in renal function) (Torres et al., 2012). During a three-year period, tolvaptan treatment

almost halved kidney growth and slowed kidney functional deterioration by a slope of -2.61 $(mg/ml)^{-1}$ per year, compared with -3.81 $(mg/ml)^{-1}$ from placebo. These exciting results were tempered by adverse effects unrelated to ADPKD in the tolvaptan group as opposed to placebo. Uncomfortable aquaresis challenged patient compliance and troubling hepatotoxicity raised concerns for possible long-term toxicity (Torres et al., 2012). A recent one-year trial, REPRISE, involved 1370 patients matching the same criteria as the previous one, and monitored liver-enzymes monthly (Torres et al., 2017). In this follow-up trial, tolvaptan similarly retarded loss of renal function for advanced PKD patients but elevated hepatic enzymes (*e.g.*, alanine aminotransferase) to the same levels observed in chronic hepatitis (Torres et al., 2017). Reassuringly, such elevated liver enzyme levels normalized upon treatment termination (Torres et al., 2017). Tolvaptan is thought to be appropriate for the subset of patients with the following

characteristics: 1- being 18-50 years old, 2- have fast disease progression, 3- have low water and/or high salt intake (Sans-Atxer and Joly, 2018). Importantly, tolvaptan and V2R antagonists display no effect on liver cysts (frequent in PKD patients) because hepatocytes do not express V2R. Tolvaptan effectiveness may attenuate over time (Torres et al., 2012), thus additional studies are required to test these parameters. Furthermore, it will be important to explore properties of other prospective V2R antagonists, including synthetic alternatives, which could potentially benefit a wider subset of PKD patients. Currently, tolvaptan represents the only therapy offered to ADPKD patients.

V2 antagonism in neurological disorders. V1aR antagonist balovaptan is being studied as a pharmacological option for ASD to modulate neurotransmitter function (Table 2). Currently used antipsychotics have shown serious side effects on lipid and glucose metabolism and increase risk of obesity, type 2 diabetes and cardiovascular disease (Correll et al., YEAR). Balovaptan was used in Improve Social Communication in Autism (VANILLA), a phase 2 clinical trial of 223 individuals with ASD. Doses of 4mg and 10mg of balovaptan improved score of the Vineland-II Adaptive Behavior Scale of ASD patients with few adverse side effects (Bolognani et al., 2019).

Synthetic AVP analogs. AVP analogs are used to activate AVP-type signaling through receptor activation. Desmopressin (Table 3) is an AVP-like compound that contains only one deaminated cysteine, and a D-arginine as opposed to its L-isomer in position 8. Desmopressin is more stable *in vivo* than AVP (Noskov et al., 2012). It binds x, y,,, receptors. X mg desmopressin administered orally, or intranasally are used to treat patients suffering with nocturnal polyuria and enuresis, i.e., involuntary urination during the night from altered daily urine production (Kamperis et al. 2017; Noskov et al., 2012). Desmopressin is with proper medical follow up.

Terlipressin preferentially binds to V1Rs (Table 3) Because of its x-times higher affinity for V1Rs than AVP, terlipressin can restore the refractory hypotension and lessen noxious hyponatremia and vascular leaks in septic shock patients with catecholamine-refractive vasodilatory shock (Noskov et al. 2012; Park et al., 2017). Administration of one/two bolus injections of 1mg terlipressin to these patients was found to increase mean arterial pressure, decrease heart rate, and improve renal function (Leone et al., 2004). With a nine-time longer half-life than AVP (50 vs. 6 min), terlipressin has sustained vasoconstriction effects that must be considered in its clinical use (Scarpati and Piazza, 2013).

Felypressin, another V1aR ligand, plays a very similar role to natural AVP. The effects of modifying structural components of AVP, were monitored by changes in mean arterial pressure following administration of felypressin, AVP, or epinephrine in rats (Cecanho et al., 2006). Note, epinephrine… what does it do? What does felypressin do? This indicates how synthetic analogues of vasopressin might potentially bring forth newer and more advanced treatments for medical interventions such as septic shock where HR must be elevated in a facilitated manner. Felypressin could be used during anesthesia to limit the hazardous hemodynamic changes seen with epinephrine (Cecanho et al., 2006). Felypressin however, has hypertensive effects at high doses and must be carefully monitored (ib.).

AVP based therapeutics. During cardiopulmonary resuscitation (CPR), the AVP and epinephrine vasoconstrictive effects could be combined to elevate the aortic pressure and stably restore coronary perfusion pressure (Kamperis et al., 2017). While epinephrine is the drug administered preferentially, AVP has the added benefit of increasing blood flow to vital organs and tissues, including skeletal muscles, skin and bowel, while simultaneously causing a vasodilatory effect on cerebral flow which improves brain blood flow (Kamperis et al., 2017).

Conclusions

Conserved in terrestrial vertebrates, vasopressins are a group of nonapeptide hormones central to maintain homeostasis and adapt to environmental and social changes in both common and species-specific ways. Characterized by an arginine in position 8, the human AVP function has been studied in the kidney, heart and brain. Several cell types produce AVP and release it in the blood stream upon stimulation to affect fluid balance and blood pressure. Because of the impermeability of the blood brain barrier, brain AVP is released centrally. While AVPir cells display sex- and individual specific distribution and localization patterns, the discovery of new AVPir is expected in the brain. Widespread and regulated expression of three main high-affinity AVP receptors, V1aR, V1bR, V2 and at least one other receptor, OTR, that can be cross-activated at high AVP concentrations, indicates that many cell types can respond to AVP signaling, depending on context. AVP affects water readsorption in the terminal region of the renal tubule, and vasoconstriction to conserve water and facilitate cardiovascular function.

While the fluid homeostasis and pressor functions of AVP are believed to have enabled transition to a terrestrial environment, neural AVP appears to be equally important for survival in that it coordinates sensory processing and behavioural modulation through the intricate connections of AVP-producing neurons in distinct brain areas. Some AVP-dependent pathways are sensitive to hormonal signaling, e.g., gonadal steroids, serotonin, and OT, which enables adaptive fine-tuning of the AVP-dependent regulation to the changing needs of a growing individual (e.g., sexual behaviour, reproduction, seasonal adaptations), and sociality (e.g., individual recognition, emotional processing, social stress, communication, parental behaviour).

Altered AVP signaling has been linked to PKD, HF and certain psychiatric conditions, including ASD, bipolar and borderline personality disorder. Thus, the AVP network constitutes an attractive pharmacological target. Receptor antagonists with distinct receptor-specificities, affinity, and half-life in vivo have been identified and are being used to modulate branches of the AVP functional network, as well as to relieve some of the neurological symptoms and ameliorate the social difficulties caused by childhood adversity in individuals with borderline personality disorder. Deciphering the mechanistic aspects of AVP signaling both in tissue- and cell-specific detail, their cross-talk and systemic integration is therefore central to achieve a more complete understanding of the AVP role in physiological and behavioural integration and to develop therapeutic strategies for PKD, CHF, ASD and other AVP-related diseases.

Author's contributions

The following authors contributed text on the topics as indicated: Nazifa Ali, Carla-Maria Avelar, Brenton Barrow, Marion Shannon Bardies on AVP in animal parental care; Giuliana Biancardi, Raminder Bindra, Lisa Bui, Minky Benedikt, Zakaria Chihab on the heart-brain axis; Tina Daigneault, Jocelyn Dault, Jeffrey Costa, Ashley Cossitt, Isa Davidson on AVP and metabolic disorders; Emie Dufour, Jonathan Dias, Nargess Farhangdoost, Sabine Khoury, Anika Forget on chronic kidney disease; Thivya Iyanker; Elias Gomah; Elie Haber, Pegah Hadavi, Claudia Hamel on AQP2 and CHF; Ansley Gnanapragasam, Alexa Fox, Maria Gentile, Myriam Gebrael, Olivia Geraci, Saad Razzaq, Ryan Scartozzi, Samantha Rhainds, Davindra Singh, Damien Robin, on AVP and water homeostasis; Christina Kalantzis, Tamar Kalostian, Sara Kamali, Elsa Kassardjian, Krissy Kontos AVP-related diseases; Danielle Mac Rae, Daniella LoScerbo, Thi Bich Uyen Le, Fleure Maurer, Yan Fang Low on AVP and kidney permeability; Sana Mazhar, Kathy Mu, Alice Nguyen, Kathy Nguyen, Joshua Oliver on AVP and circadian rhythms; Chelsea Osborne-Laroche, Emilie Parolin, Jane H.W. Park, Leah Sarah Peer, Kahlila Paul-Cole on AVP in heart disease; Jessica Porras-Marroquin, Charles Plaisir, Simran Prasad, Margaux Philippon, Ramsarun Rewaprasad on AVP, brain and behaviour. Laila Toro, Nastaran Soroori-Motlagh, Kiri Stern, Sajad Soleimani Fard, Maxim Soroko on AVP, CHF and treatments; Aaliyah Weekes, Allison Wisniewski, Claudia Waddingham, Modibo Toure', Sarah Trepanier-Chicoine, Stephanie Tran on OT roles in ASD; Samantha Sparapani wrote text on AVP synthesis and processing, pharmacology and edited the manuscript; Cassandra Millet-Boureima wrote the section on AVP and PKD, curated and edited the manuscript; Joshua Oliver wrote the section on heart disease, contributed to the pharmacology section, and edited the manuscript; Kathy Mu, Tamar Kalostian and Pegah

Hadavi respectively contributed to the sections on AVP and brain function, pharmacology and to manuscript editing; Chiara Gamberi planned the review contents, directed the assignment and editing activities, co-wrote the section on AVP neurobiology, and edited the manuscript.

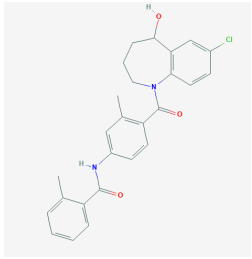
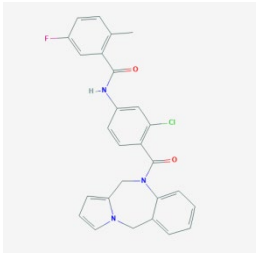
Funding statement

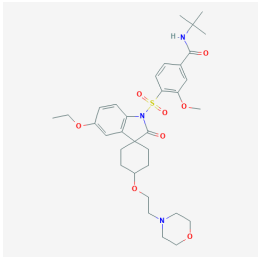
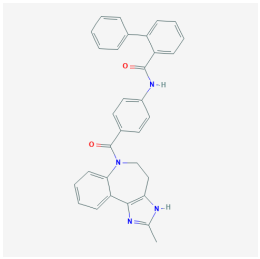
As an educational project, this manuscript was curated on a volunteer basis from each and all authors, including the corresponding. Therefore, no funding was utilized.

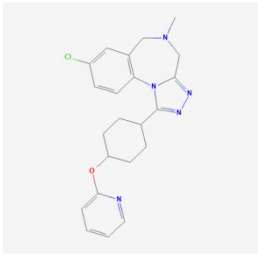
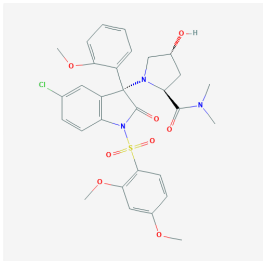
Acknowledgments

We are grateful to Concordia University Biology Librarian Katharine Hall for support and assistance during this project; Dr. Almira Kurbegovich for critical comments to the manuscript, Prof. William D. Lubell for the vasopressin structure.

Table 2. AVP Antagonists

AVP Receptor Antagonists	Chemical structure	Chemistry	Mechanism of Action	Route of Administration	Clinical Progress	References
Tolvaptan		Empirical formula:$C_{26}H_{25}ClFN_3O_2$ Chemical nomenclature: *N* [4-(7-chloro-5-hydroxy-2,3,4,5-tetrahydro-1-benzazepine-1-carbonyl)-3-methylphenyl]-2-methylbenzamide	Binds to V2R with 30-fold higher affinity than V1aR *in vivo*. Reduces water reabsorption within the renal collecting ducts and promotes aquaresis.	Oral	Entered clinical trial phase 3 as of 2012 and has been shown to reduce the rate of renal function decline in ADPKD patients (TEMPO, REPRISE trials).	Miyazaki et al., 2007; Finley al., 2008; Torres et al., 2012; Waller et al., 2013; Rangarajan et al., 2014 ADD the TEMPO and REPRISE trial references
Lixivaptan		Empirical formula:$C_{27}H_{21}ClFN_3O_2$ Chemical nomenclature: (*N*-[3-chloro-4-(6,11-dihydropyrrolo[2,1-c][1,4]benzodiazepine-5-carbonyl)phenyl]-5-fluoro-2-methylbenzamide	Binds V2R with 100-fold higher affinity than V1aR. Prevents translocation and localization of AQP2 to the renal collecting ducts and promotes aquaresis.	Oral	Will enter clinical trial phase 3 in 2021 for ADPKD. Further study to serve in treating severe cases of hyponatremia and CHF.	Albright et al., 1998; Finley et al., 2008; Ku et al., 2009; De Mise et al., 2019

Satavaptan		Empirical formula: $C_{33}H_{45}N_3O_8S$ Chemical nomenclature: 1-[4-(N-tert-butylcarbamoyl)-2-methoxybenzene sulfonyl]-5-ethoxy-3-spiro-[4-(2 morpholinoethoxy)cyclohexane] indol2-one	V2R selectivity is 112 times greater than for V1aR. Prevents AVP binding to receptors kidney medullo-papillary membranes and thus lowers fluid retention. Is this different from the other V2R antagonists?	Oral	Clinical trial phase 3 as of 2008. Demonstrated to improve cases of ascites. Renormalizes Na^+ levels in hyponatremic patients diagnosed with liver cirrhosis.	Gines et al., 2008; Finley et al., 2008; Farah et al., 2010
Conivaptan		Empirical formula: $C_{32}H_{26}N_4O_2$ Chemical nomenclature: *N*-[4-(2-methyl-4,5-dihydro-3*H*-imidazo[4,5-d][1]benzazepine-6-carbonyl)phenyl]-2-phenylbenzamide	High affinity for both V1aR and V2R. V1aR: reduces Ca^{2+} mobilization and kinase activity within cardiac tissue and reduces myocardium hypertrophy. V2R: inhibits AQP2 translocation to the apical membrane of the collecting duct and thus lowers water reabsorption. Aquaretic?	Oral & Intravenous	Clinical trial phase 2 as of 2004. Tested to treat cases of hyponatremia and decompensated HF.	Ali et al., 2007; Finley et al., 2008

Balovaptan		Empirical formula: $C_{22}H_{24}ClN_5O$ Chemical nomenclature: 8-chloro-5-methyl-1-(4-pyridin-2-yloxycyclohexyl)-4,6-dihydro-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine	Selective V1aR antagonist. Improved Vineland II adaptive behavior scales.	Oral	Entered clinical trial phase 3 as of 2018 and is undergoing testing to correct behavioral complications associated with ASD (VANILLA trial).	Bolognani et al.,YEAR ; Schnider et al., 2020
Nelivaptan		Empirical formula: $C_{30}H_{32}ClN_3O_8S$ Chemical nomenclature: (2*S*,4*R*)-1-[(3*R*)-5-chloro-1-(2,4-dimethoxyphenyl)sulfonyl-3-(2-methoxyphenyl)-2-oxoindol-3-yl]-4-hydroxy-*N*,*N*-dimethylpyrrolidine-2-carboxamide	Selectively binds V1bR. Normalizes hypersecretion of ACTH in presence of stress stimuli.	Oral	Exhibited potential as an anxiolytic and anti-depressant. Entered clinical trial phase 2 as of 2008.	Serradeil-le-Gal et al., 2005; Schule et al., 2009

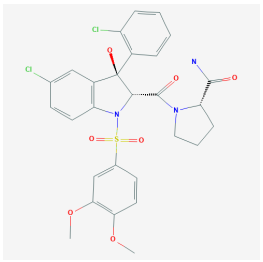
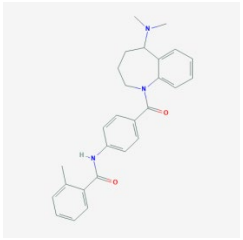
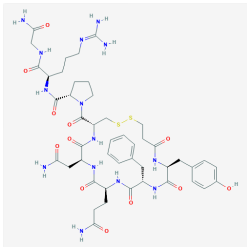
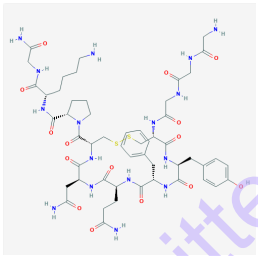
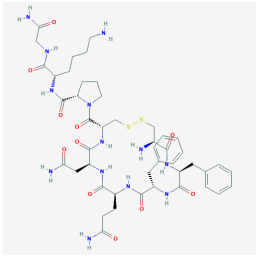
Relcovaptan		Empirical formula: $C_{28}H_{27}Cl_2N_3O_7S$ Chemical nomenclature: (2S)-1-[(2R, 3S)-5-chloro-3-(2-chlorophenyl)-1-(3,4-dimethoxyphenyl) sulfonyl-3-hydroxy2H-indole-2-carbonyl] pyrrolidine-2-carboxamide	Selectively binds V1aR. Promotes vasodilatation of the vascular bed.	Oral	Promise in treating Raynaud's syndrome, regulation of uterine contractions in preterm labor. Currently in clinical phase 2 trial.	Steinwall et al., 2005; Decauz et al., 2008
Mozavaptan		Empirical formula: $C_{27}H_{29}N_3O_2$ Chemical nomenclature: *N*-[4-[5-(dimethylamino)-2,3,4,5-tetrahydro-1-benzazepine-1-carbonyl]phenyl]-2-methylbenzamide	V2R antagonist. Inhibits the antidiuretic action of AVP. Aquaretic?	Oral	Tested to treat ectopic ADH syndrome and CHF. Approved in Japan since 2006 to treat severe cases of hyponatremia.	Decaux et al., 2009; Yamaguchi et al., 2011

Table 3. AVP Analogs

AVP Analogs	Chemical structure	Chemistry	Mechanism of Action	Route of Administration	Clinical Progress	References
Desmopressin		Empirical formula: $C_{46}H_{64}N_{14}O_{12}S_2$ Chemical nomenclature: (2*S*)-*N*-[(2*R*)-1-[(2-amino-2-oxoethyl)amino]-5-(diaminomethylideneamino)-1-oxopentan-2-yl]-1-[(4*R*,7*S*,10*S*,13*S*,16*S*)-7-(2-amino-2-oxoethyl)-10-(3-amino-3-oxopropyl)-13-benzyl-16-[(4-hydroxyphenyl)methyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentazacycloicosane-4-carbonyl]pyrrolidine-2-carboxamide	Selectively binds V2R. Induces the translocation of AQP2? to the apical membrane of the renal collecting duct and subsequent water reabsorption.	Oral & Intranasal	Phase 3 clinical trial FOR WHAT? as of 2008. Currently used to treat nocturia.	Noskov et al., 2012; Kamperis et al. 2017
Terlipressin		Empirical formula: $C_{52}H_{74}N_{16}O_{15}S_2$ Chemical nomenclature: (2*S*)-1-[(4*R*,7*S*,10*S*,13*S*,16*S*,19*R*)-19-[[2-[[2-[(2aminoacetyl)amino]acetyl]amino]acetyl]amino]-7-(2-amino-2-oxoethyl)-10-(3-amino-3-oxopropyl)-13-benzyl-16-[(4-hydroxyphenyl)methyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentazacycloicosane-4-carbonyl]-*N*-[(2*S*)-6-amino-1-[(2-amino-2-oxoethyl)amino]-1-oxohexan-2-yl]pyrrolidine-2-carboxamide	Displays affinity for both V1aR and V2R. V1aR: induces splanchnic and renal vasoconstriction, thus leading to reduced portal pressure. V2 R: increases AQP2? density in the renal apical membrane leading to greater water retention.	Intravenous	Phase 3 trials as of 2019. FOR WHAT? Used to control variceal bleeding in cases of portal hypertension and acute kidney injury.	Leone et al., 2004; Sarin et al., 2011 Scarpati et al., 2013

Felypressin		Empirical formula: $C_{46}H_{65}N_{13}O_{11}S_2$ Chemical nomenclature: 2*S*)-*N*-[(2*S*)-6-amino-1-[(2-amino-2-oxoethyl)amino]-1-oxohexan-2-yl]-1-[(4*R*,7*S*,10*S*,13*S*,16*S*,19*R*)-19-amino-7-(2-amino-2-oxoethyl)-10-(3-amino-3-oxopropyl)-13,16-dibenzyl-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentazacycloicosane-4-carbonyl]pyrrolidine-2-carboxamide	Selectively interacts with the V1aR. Induces smooth muscle contraction within the vascular bed.	Intramuscular	Primarily used as a hemostatic to normalize blood pressure in hypertensive patients.	Olgart et al., 1977; Cecanho et al., 2006; Bronzo et al., 2012