

Review

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Copeptin (CTproAVP), a new tool for understanding the role of vasopressin in pathophysiology

Abstract: Arginine vasopressin (AVP) plays a key role in many physiologic and pathologic processes. The most important stimulus for AVP release is a change in plasma osmolality. AVP is also involved in the response and adaptation to stress. Reliable measurement of AVP is hindered by several factors. Over 90% of AVP is tightly bound to platelets, and its estimation is influenced by the number of platelets, incomplete removal of platelets or pre-analytical processing steps. Copeptin (CTproAVP), a 39-aminoacid glycopeptide, is a C-terminal part of the precursor pre-provasopressin (pre-proAVP). Activation of the AVP

system stimulates CTproAVP secretion into the circulation from the posterior pituitary gland in equimolar amounts with AVP. Therefore CTproAVP directly reflects AVP concentration and can be used as a surrogate biomarker of AVP secretion. In many studies CTproAVP represents AVP levels and its behavior represents changes in plasma osmolality, stress and various disease states, and shows some of the various physiologic and pathophysiologic conditions associated with increased or decreased AVP. Increased CTproAVP concentration is described in several studies as a strong predictor of mortality in patients with chronic heart failure and acute heart failure. Autosomal polycystic kidney disease (ADPKD) patients have both central and nephrogenic defects in osmoregulation and CTproAVP balance. A possibility raised by these clinical observations is that CTproAVP may serve to identify patients who could benefit from an intervention aimed at countering AVP.

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Introduction

Arginine vasopressin (AVP), also known as antidiuretic hormone, plays a key role in many physiologic and pathologic processes. Both AVP and its closely related peptide oxytocin are highly conserved and appear to precede the divergence of vertebrate and invertebrate families. The main role of AVP is to induce water conservation by the kidney, thus contributing to osmotic and cardiovascular

homeostasis. It also has hemostatic, endocrine and central nervous effects.

AVP is a nine-amino acid peptide with a disulfide bridge between two cysteine amino acids. It is produced primarily in the magnocellular neurons of the hypothalamus, and is stored and secreted by granules within the posterior lobe of the pituitary, primarily as a response to high plasma osmolality, low plasma volume, and/or low blood pressure. To a lesser extent, it is also produced in other tissues including the sympathetic ganglia, adrenal glands, and the testis [1].

The synthesis of AVP involves precursor peptides (pre-proAVP and proAVP) that are enzymatically cleaved by a four-enzyme cascade into the components vasopressin, copeptin (CTproAVP) and neurophysin II. These three cleavage products are all released into the circulation in equal ratios.

The most important stimulus for AVP release is a change in plasma osmolality. This occurs via peripheral receptors whose afferent stimuli ascend via the vagus nerve through the medulla to the hypothalamic nuclei. A small change, of even 1%, in plasma osmolality is sufficient to change AVP concentration. Normal AVP concentrations in healthy individuals are between 1 and 5 pg/mL.

AVP is also an important factor of the response and adaptation to stress. Stressful stimuli evoke complex endocrine, autonomic, and behavioral responses that are extremely variable and specific depending on the type and nature of the stressors. Adaptation to stress stimuli, either acute or chronic, largely depends on the activation of the hypothalamic-pituitary-adrenal (HPA) axis and the catecholaminergic system [2]. Appropriate regulatory control of the HPA stress axis is essential to health and survival. The outflow of the HPA axis is therefore a summation of integrated inputs from several forebrain regions. In the hypothalamic areas, the cascade is in turn chiefly regulated by the corticotropin releasing hormone (CRH) and AVP. Hyperactivation of the sympathetic nervous system (SNS) also plays a partnership role in the body's response to both acute and chronic stress [3]. AVP is particularly produced in response to stress or acute life-threatening events (physical stress conditions), and is involved in changes in blood pressure and volume.

AVP produced under these circumstances greatly exceeds the physiological range with exponential levels 100- or 1000-fold times the normal concentration. AVP exerts its effects on three different receptors. The V1a receptors mediate strong arteriolar vasoconstriction and are localized primarily on vascular smooth muscle cells, hepatocytes, platelets, and cells in the brain and uterus. V2 receptors mediate antidiuretic effects, and are highly

expressed in the kidneys, and in endothelial cells and vascular smooth muscle. Upon stimulation, V2 receptors activation is associated with an increased intracellular level of cAMP, which in turn triggers the insertion of aquaporin-2 water channels in the apical membrane of tubular cells of the distal nephron, leading to water reabsorption into the interstitium. V3 receptors (also known as V1b receptors), found in the anterior pituitary, brain, pancreas, and heart are involved in ACTH secretion, neuromodulation, insulin synthesis and release, temperature and memory control [1, 4–7].

The plasma half-life of AVP is quite short, of about 5–20 min. Clearance of AVP usually occurs by the kidney or liver, with renal clearance estimated at approximately 50%–70% of total clearance [1]. AVP can also be degraded by several endothelial and circulating endo- and amino peptidases. Clearance is proportional to plasma levels of AVP and reaches 600 mL/min at a concentration of 10 pg/mL in humans. Urinary clearance is about 5% of the total clearance [1].

AVP action has been linked to liver glycogenolysis via V1a receptors and insulin and glucagon secretion via V1b receptors. AVP levels are markedly increased in patients with poorly controlled diabetes mellitus [8] and AVP infusion leads to increased blood glucose levels in healthy subjects [9]. AVP predicts diabetes mellitus independently of a broad range of established diabetes risk factors [10].

Finally, AVP has been suggested to regulate hypermetabolic pathways of fat in V1a receptor knockout mice [11] and promote thermogenic adipokines in brown adipose tissue [12], which imply a potential role of the AVP system in the regulation of energy expenditure and balance which, in turn, may interfere with the water balance.

CTproAVP (copeptin) in physiological conditions

Reliable measurement of AVP is hindered by several factors. Over 90% of AVP is tightly bound to platelets, and its estimation is influenced by the number of platelets, incomplete removal of platelets or pre-analytical processing steps [4]. In addition, AVP is also highly unstable in isolated plasma – even when stored at –20 °C.

CTproAVP (copeptin), a 39-aminoacid glycopeptide, is a C-terminal part of the precursor pre-provasopressin (pre-proAVP). Activation of AVP system stimulates CTproAVP secretion into the circulation from the posterior pituitary gland in equimolar amounts with AVP. Therefore, CTproAVP directly reflects AVP concentration and can be

used as surrogate biomarker of AVP secretion. Even mild to moderate stress situations contribute to the release of CTproAVP. These reasons have led to several different lines of research in various disease states.

To overcome some of these disadvantages, CTproAVP can be used as a surrogate for AVP since it is very stable in plasma and during storage. Unlike AVP, it does not require any pre-analytical processing and can be easily determined with several manual and automated assays.

A large study using healthy volunteers recruited from a local heart failure screening showed that the median CTproAVP levels are slightly, but significantly higher in males compared to females [4.3 (0.4–44.3) vs. 3.2 (1.0–14.8) pmol/L]. There is a stronger correlation of CTproAVP with the estimated glomerular filtration rate (eGFR) in males compared to females and left atrial size correlated with higher CTproAVP levels [13].

Levels of CTproAVP are not strongly correlated with age [13, 14], however, strenuous exercise causes a moderate increase in CTproAVP [14].

Studies have shown that CTproAVP mirrors AVP levels under diverse physiologic and pathophysiologic conditions such as changes in plasma osmolality, stress and various disease states [15–17]. The measurement of CTproAVP can be used to reflect AVP concentrations as it is produced in equimolar amounts as AVP and released into the circulation at the same time as AVP secretion [15].

Vasopressin and CTproAVP in pathogenetic conditions

Diabetes insipidus

Diabetes insipidus (DI) is a clinical syndrome characterized by polyuria due to a defect in the urinary concentrating mechanism [18]. There is also an associated compensatory polydipsia. The prevalence in the general population is estimated at 1 per 25–35,000. The syndrome comprises three main types central, nephrogenic and gestational, and a related syndrome, primary polydipsia [19].

Central (neuro-hypophyseal) DI is associated with impaired production or secretion of AVP, such as in pituitary injury after head trauma or surgery. It is estimated that 20%–30% of pituitary operations are associated with transient central DI, and that 2%–10% lead to permanent disease [20, 21].

Central DI is also sometimes observed in course of infection or malignancy. The other type of central DI is due to a very rare congenital condition, i.e., as familial

neuro-hypophyseal DI associated with autosomal dominant, recessive or X-linked recessive mutation of the AVP gene.

Nephrogenic DI is characterized by impaired AVP-induced water adsorption [19]. Acquired nephrogenic DI is most commonly associated with electrolyte abnormalities (such as hypokalemia or hypercalcemia) or the therapeutic use of drugs, such as lithium or cisplatin.

Gestational DI, is due to an increased degradation of AVP from vasopressinase, a placental enzyme [22, 23]. Although it is not very commonly observed, it can be under diagnosed, as polyuria is considered normal during pregnancy.

DI should be differentiated from primary polydipsia. The latter differs from DI as it is not associated with variants of AVP secretion or activity – but rather from excessive fluid intake over extended periods of time. However, CTproAVP may help in the differential diagnosis of primary polydipsia and DI, although further studies are needed in the area.

An accurate differentiation of the underlying pathology is necessary for effective treatment of DI. If the patient has central diabetes insipidus, the AVP concentration will not increase – even though there has been a significant decrease in body weight or increase in plasma osmolality. However, if the patient has nephrogenic DI, AVP will appropriately increase in parallel to dehydration status progression and the increase in plasma osmolality. Desmopressin (an exogenous synthetic vasopressin analog) is commonly administered as a challenge after water deprivation to see if there is a further change in urine osmolality.

CTproAVP has been used in several recent studies as a novel approach for the diagnosis of DI [24–26]. This method evaluates osmotically stimulated CTproAVP after an 8-h water withdrawal period. The first blood sample is able to aid in the distinction between central complete and nephrogenic DI by comparing the plasma levels of CTproAVP. Concentrations of CTproAVP that are <2.6 pmol/L indicate central DI complete whereas concentrations >20 pmol/L indicate nephrogenic DI. Patients with intermediate values between 2.6 and 20 pmol/L undergo a further 8 h of fluid deprivation.

A CTproAVP index is derived by the following equation:

$$\text{Delta CTproAVP [8–16 h]} \times 1000 \text{ [pmol/L/mmol/L]} \\ \text{S-Na}^+ \text{ [16 h]}$$

Patients with a CTproAVP index of <20 are classified as having partial central DI whereas patients with values >20 have primary polydipsia [16].

Hyponatremia and SIADH (SIAD)

Hyponatremia, defined as serum sodium levels <135 mmol/L [27], is the most frequent electrolyte disorder occurring in 15%–30% of hospitalized patients. Severe hyponatremia is often defined as levels of serum sodium <125 mmol/L and represents 1%–4% of patients. However, the incidence can rise dramatically for certain patient groups. For example, institutionalized geriatric patients have incidences of 7%–53% [28] and it is estimated that as many as 40%–75% of patients have hospital-acquired hyponatremia.

Although many cases are asymptomatic, acute severe hyponatremia is associated with substantial morbidity and mortality risk. Treatment is also complicated, as overly rapid correction can lead to severe permanent neurological deficits and death. Even mild chronic hyponatremia is associated with clinical consequences, such as gait disturbances and falls, increased risk of fractures and reduced bone mineral density and attention deficits [27, 29].

A recent meta-analysis showed that a moderate reduction of serum sodium concentration is associated with an increased risk of death in different pathologic conditions [30].

The syndrome of inappropriate antidiuretic hormone secretion (SIADH) refers to disorders related to water and sodium balance characterized by the impairment of urinary dilution and hypotonic hyponatremia in the absence of renal disease or other identifiable non-osmotic stimuli known to activate the release of AVP [31]. It is a frequent cause of hyponatremia in the clinical setting with a wide range of clinical manifestations, ranging from asymptomatic to life threatening.

The underlying pathological basis for SIADH is an absolute increase in body water in relation to sodium pool due to osmoregulatory dysfunctions. SIADH can be further subdivided according to the different underlying osmoregulatory dysfunctions. Type A, is characterized by high basal AVP levels with random fluctuations in the hyponatremic state. This type of SIADH is commonly associated with tumors and is due to excessive AVP secretion. Type B is also characterized by high AVP (but with levels often lower than type A). Type B is often related to injury of the neuro-hypophysis causing generalized osmoregulatory dysfunction. Type C is thought to be due to a “downward reset” of the osmoregulatory system, while Type D, in which AVP is undetectable, has been associated with mutation in V2 receptors [32]. This discovery led to the proposal to rename SIADH with the new acronym SIAD, for syndrome of inappropriate antidiuresis.

Endogenous conditions that can cause SIAD include: 1) conditions that can increase hypothalamic (eutopic)

production of AVP; 2) conditions associated with ectopic production (such as malignancies); 3) potentiation of AVP effects; and 4) nephrogenic syndrome of inappropriate antidiuresis (NSIAD), which is characterized by gain-of-function mutations of the V2 receptor gene.

SIAD can occur in different pathologic conditions, such as infections, thrombosis or subarachnoid or subdural hemorrhage, neuropsychiatric disorders, various cerebral diseases (HIV, Guillan-Barre’, multiple sclerosis), pulmonary diseases and severe nausea or post-surgical pain. SIAD can be also induced by many drugs, such as chemotherapeutics, antipsychotics, antidepressants, opiates, and non-steroidal anti-inflammatory drugs.

CTproAVP has also been used in the differential diagnosis of hyponatremia. Fenske et al. found that while isolated measurements of CTproAVP had only limited value for identification of patients with polydipsia, a combination of CTproAVP and urinary sodium gave superior performance to the reference standard in discriminating volume depleted from normovolemic hyponatremic disorders [16].

Unlike true hyponatremia, *pseudohyponatremia* is a condition represented by low serum sodium concentration resulting from volume displacement by massive hyper-lipidemia or hyper-proteinemia, or by hyperglycemia. Therefore, reduced sodium concentration in pseudo-hyponatremia does not entail a true hypotonic disorder. The correct identification of pseudo-hyponatremia is essential to avoid unnecessary and dangerous treatments aiming at restoring normal sodium values.

Heart failure

Heart failure is commonly associated with hyponatremia, and is also characterized by increased concentrations of basal AVP and CTproAVP [33–35]. In addition to plasma osmolality, non-osmotic factors, such as intra-cardiac pressures, intra-arterial pressures, angiotensin II, pain, and adrenergic (α_2) central nervous stimuli can also influence AVP secretion [36]. Although non-osmotic mechanisms do not usually play an important role in healthy individuals, edematous states are characterized by a shift in regulation towards predominance of non-osmotic over the physiological osmotic stimuli. In addition, AVP through its V1 and V2 receptor-mediated effects, seems to contribute to the progression of left ventricular dysfunction by worsening systolic and diastolic wall stress and by stimulating both ventricular hypertrophy and myocardial remodeling.

Several studies have shown that increased CTproAVP concentration is a strong predictor of mortality in patients

with chronic heart failure, acute heart failure and heart failure caused by acute myocardial infarction [33, 37–39].

CTproAVP values are higher in non-survivors compared to survivors for patients with advanced heart failure, as well as acute heart failure. The typical range of CTproAVP for patients with chronic or acute heart failure is between 10 and 50 pmol/L compared to the normal values of 4–7 pmol/L. These clinical observations and the previously discussed physiological and pathophysiological effects suggest that CTproAVP may be an interesting prognostic biomarker to be measured and monitored in patients with heart disease.

CTproAVP levels predict mortality in outpatients with chronic heart failure independently from clinical variables, such as plasma sodium. It also predicts the combined endpoint of hospitalization or death independent of NTproBNP [38]. Results from the BACH study also showed that patients with elevated CTproAVP and the lowest levels of sodium had the highest mortality at 90 days. Combined end points of mortality, readmissions and emergency department visits were also associated with higher CTproAVP [37, 40].

If patients at high short-term mortality risk are prospectively selected, adequate power for interventional studies may be obtainable with much smaller trials [39].

Renal disorders

Accruing evidence over the last several years suggests that AVP plays an important role in the initiation and progression of chronic kidney disease, kidney transplantation and as well as specific roles in disorders, such as autosomal polycystic kidney disease (ADPKD) [5, 41–43].

In population-based cohorts, the annual decline in eGFR has been inversely correlated to 24-h fluid intake and urine volume. Independent studies also have correlated CTproAVP concentration and albuminuria with renal decline in kidney transplant recipients [44].

Although there are many, still open, questions regarding the underlying pathophysiology, there is strong evidence that AVP modifies vascular tone in renal microvessels. AVP acting via the V2 receptors may also reduce the efficiency of sodium and urea excretion and thus increase glomerular filtration rate thus imposing increased energetic demands on the kidney. In addition, AVP promotes mitogenesis and proliferation of mesangial cells via the V1 receptors, which mediate AVP-induced cell contraction. In part, this may also be due to AVP stimulation of endothelin-1, a vasoactive peptide with mitogenic effects. Studies have shown that addition of AVP to cultured mesangial

cells increases in a dose dependent manner the synthesis and release of matrix proteins, such as type I and IV collagen, fibronectin and transforming growth factor. All these may interfere with the normal contractile, immunologic and endocrine actions of mesangial cells, which are crucial for the maintenance of glomerular health [5]. AVP may be especially important in ADPKD.

CTproAVP is correlated with the severity of the disease and recent evidence suggests that ADPKD patients have both central and nephrogenic defects in osmoregulation and CTproAVP balance [45–47]. AVP has a direct influence on cysts, by stimulating the formation of cAMP, a potent stimulator of cyst growth, particularly of cysts that originate from the distal nephron segments that express V2 receptors. The CRISP study showed that ADPKD patients with relatively well-preserved renal function that had higher 24-h urine osmolality at baseline were at higher risk of a faster decline of eGFR. These results confirm pre-clinical and experimental findings [48] that blocking the endogenous activity of AVP on V2R might counteract the cystic phenotype. Tolvaptan, a selective V2R-antagonist, slowed the increase in total kidney volume and the progression toward kidney failure in patients with ADPKD but was associated with a higher rate of adverse events in the TEMPO 3:4 trial [48]. High water intake decreases plasma and urine osmolality and suppresses AVP release from CNS. As a result, there is no or little renal V2R stimulation by AVP, reduced cAMP synthesis and slower cyst growth. This approach was successful in reducing the cystic phenotype in experimental models [49].

In pilot studies in humans, a short course of high water diet was effective in reducing urinary cAMP in ADPKD patients [50, 51]. An intriguing possibility raised by clinical observations is that CTproAVP may serve to identify patients who may benefit from an intervention aimed at countering AVP.

Summary

The main role of AVP is to induce water conservation by the kidney, and contribute to osmotic and cardiovascular homeostasis. Reliable measurement of AVP is hindered by several factors. CTproAVP can be used instead as a surrogate to provide insight on AVP, as it is produced at the same time, has high plasma stability and is easy to measure.

In many studies CTproAVP represents AVP levels and its behavior represents changes in plasma osmolality, stress and various disease states, showing some of the various physiologic and pathophysiological conditions associated with increased or decreased AVP.

Several studies have shown that increased CTproAVP concentration is a strong predictor of mortality in patients with chronic heart failure, acute heart failure and heart failure caused by acute myocardial infarction. ADPKD patients have both central and nephrogenic defects in osmoregulation and CTproAVP balance.

CTproAVP represents a prognostic biomarker for acute illness and has been shown to correlate with clinical outcome in various critical diseases. The evaluation of chest pain patients is critical and involves several clinical specialities. The utility of combining CTproAVP to troponin may provide additional value in ruling-out or -in patients in life-threatening emergencies [52].

For a biomarker to be considered useful in clinical practice [53], four criteria should be met: 1) accuracy, i.e., the ability to reliably identify individuals at excessive risk of adverse clinical outcome of interest; 2) simplicity, such as ease of measurement; cost, as biomarkers should be reasonably cost-effective; 3) relevance of the information provided by the biomarker, as this information should be additive to that conveyed by established risk factors; and finally 4) biomarkers need to be tested in a proper randomized clinical trial where a clinical policy based on the biomarker of interest is compared with the traditional, standard clinical policy applied in the patients' population of interest.

Larger clinical trials to evaluate the feasibility and long-term effects of this approach are currently ongoing. An intriguing possibility raised by these clinical observations is that CTproAVP may serve to identify patients who may benefit from an intervention aimed at countering AVP.

The open question on the use of CTproAVP in the diagnosis and treatment of hyponatremia [54] seems to receive a promising answer.

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