Chapter 1
Introduction
This thesis consists of a series of studies related to the hormone vasopressin. Vasopressin is recognized for its considerable role in human physiology with widespread effects on various regulatory processes. However, in addition to its imperative role in water homeostasis, it has also been associated with progression of kidney disease, especially autosomal dominant polycystic kidney disease.

**The importance of water homeostasis**

One of the main constituents of the human body is water. It makes up about 50% of the total lean body weight. Water is distributed amongst cells, interstitial tissue and blood plasma. It moves freely through the barriers between these compartments (e.g. the cell membrane and the vascular wall) until an equilibrium in osmolality is reached, which is around 280 mOsm/kg. This set-point is preserved carefully by regulatory mechanisms, in order for the bodily functions to work properly. These mechanisms are fundamental, given that the total water content of the body is not static, but changes from moment to moment. There are losses of water through sweat, feces and urine, and gains through intake of food and fluids. The kidneys play a key role in maintaining the equilibrium. They have the capacity to reabsorb significant amounts of water from the tubular lumen, thereby reducing urinary output and returning water to the circulation, or conversely, to eliminate an excess of water by producing diluted urine. In addition to fine-tuning of water excretion by the kidneys, thirst sensation is vital to secure adequate water intake. The importance of a tight regulation of osmolality is demonstrated by the symptoms that develop when these mechanisms fail. An increase or decrease in osmolality, if not met with a direct and adequate response, causes water to be redistributed over the compartments. This causes cells to shrink or swell, thereby impairing their function. Especially brain neurons are easily damaged by these volume changes.

**Vasopressin regulates water reabsorption by the kidneys**

The kidneys have the ability to respond immediately to changes in plasma osmolality. The stimulus that governs water handling by the kidneys is provided by a small peptide hormone, called vasopressin. Because vasopressin strongly promotes water reabsorption, it is also known as the antidiuretic hormone.

Vasopressin is synthesized by supraoptic and paraventricular cells in the hypothalamus and is thereafter transported via axonal pathways to the posterior pituitary, from where it is released into the circulation. A rise in plasma osmolality, registered by osmotic receptor cells within the hypothalamus, is the most potent stimulus for vasopressin secretion. In addition, a significant drop of blood volume or decrease in blood pressure can also trigger its release. Vasopressin is carried via the bloodstream to the kidney, where it binds to a cell membrane receptor on the collecting duct tubules, the V2 receptor. Activation of
these receptors induces translocation of aquaporin-2 channels from the cytoplasm to the apical cell membrane. Aquaporin-2 is a protein belonging to a family of water channels. The cell membrane of the tubular cells is impermeable to water without these channels. Through aquaporin-2, water starts to flow along an osmotic gradient from the lumen of the collecting duct into the interstitial tissue and capillaries of the renal medulla. Thus, water is brought back into the circulation. After plasma osmolality returns to its normal range, vasopressin secretion by the pituitary is halted, aquaporin channels are degraded, and water reabsorption is ceased.\textsuperscript{9}

Without the antidiuretic effect of vasopressin, a significant clinical disease develops. This condition, called diabetes insipidus, illustrates the significance of vasopressin. Diabetes insipidus can be caused by a central deficit, when vasopressin is not released from the posterior pituitary, or by insensitivity of the kidney cells to the vasopressin stimulus. This can give rise to a urinary output of up to 12 liters a day. Osmolality is maintained by water intake only, which is procured by an unrelenting thirst.\textsuperscript{10,11}

**Vasopressin influences the osmolar gradient in the inner medulla of the kidney**

It is important to note that vasopressin does not only promote reduction of urine volume by regulating the permeability of the tubular cells, it also regulates complementary processes that are imperative to achieve antidiuresis. The presence of an osmotic gradient in the interstitial tissue is essential to draw water out of the tubular lumen into the circulation. This gradient is established by urea, which is present at high concentrations in the medulla of the kidney. This high concentration is attained by a cycling of urea through the nephron. Countercurrent exchange of urea from the lumen of the tubules into the adjacent vessels through specific urea transporters results in the accumulation of urea in the inner medulla.\textsuperscript{12} Vasopressin is essential for this build-up of urea concentration. V2 receptor stimulation is required for the insertion of urea transporters in the collecting duct cell membrane, thereby enabling urea to diffuse to the interstitial tissue.\textsuperscript{13,14}

Thus, vasopressin is required for accomplishing the maximal concentrating capacity of the kidneys. It is essential, both to render the tubular cells permeable for water as well as to stimulate the build-up of an osmotic gradient. With these two mechanism intact, urinary output is effectively reduced to a minimum required to excrete waste products.

**Three types of vasopressin receptors**

In addition to its antidiuretic effects, vasopressin is thought to mediate a variety of other physiological processes. The notion that vasopressin’s role is not limited to the collecting duct, but indeed more far-reaching, originates from the widespread presence of vasopressin receptors on different cell types throughout the body. There are three
receptor subtypes that use vasopressin as their ligand, respectively named the V1, V2 and V3 receptor\textsuperscript{15,16} The structural build of these three types of receptors is fairly comparable, with a sequence homology of 35 to 50%. The main effects of vasopressin mediated through the V2 receptor have been described in the previous paragraphs. This antidiuretic effect is considered the principal function of vasopressin. The clinical significance of the other receptors is less clearly defined. A summary of the various actions of vasopressin through activation of V1 and V3 receptors (also called V1a and V1b receptors, respectively) is presented in Table 1.

Table 1. Suggested vasopressin actions mediated through the V1 and V3 receptor subtypes

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Side of action</th>
<th>Effect</th>
</tr>
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<tbody>
<tr>
<td>V1</td>
<td>Vascular smooth muscle cells</td>
<td>Increasing blood pressure by vasoconstriction\textsuperscript{17-19}</td>
</tr>
<tr>
<td></td>
<td>Platelets</td>
<td>Activation of platelet aggregation\textsuperscript{20}</td>
</tr>
<tr>
<td></td>
<td>Collecting duct cells of the kidney</td>
<td>Promoting diuresis, thereby antagonizing the V2 effects\textsuperscript{8}</td>
</tr>
<tr>
<td></td>
<td>Macula densa of the kidney</td>
<td>Increasing blood pressure by induction of renin secretion via increase of nitric oxide and prostaglandin\textsuperscript{21}</td>
</tr>
<tr>
<td></td>
<td>Intercalated cells of the kidney</td>
<td>Increasing of H\textsuperscript{+} secretion and bicarbonate reabsorption, thereby changing the effect of aldosterone on acid-base homeostasis\textsuperscript{22-24}</td>
</tr>
<tr>
<td></td>
<td>Cortex of the adrenal gland</td>
<td>Increasing glucocorticoid activity by stimulating cortisol secretion and increasing blood pressure by stimulating aldosterone secretion\textsuperscript{25}</td>
</tr>
<tr>
<td></td>
<td>Liver cells</td>
<td>Increasing blood glucose levels by enhancing glycogenolysis\textsuperscript{26}</td>
</tr>
<tr>
<td></td>
<td>Central nervous system, area postrema</td>
<td>Increasing blood pressure by lowering the threshold of the baroreceptor reflex\textsuperscript{16}</td>
</tr>
<tr>
<td></td>
<td>Central nervous system</td>
<td>Negatively affecting the resilience to stress, increasing anxiety and depression\textsuperscript{27,28}</td>
</tr>
<tr>
<td>V3</td>
<td>Anterior pituitary gland</td>
<td>Increasing glucocorticoid activity by potentiating the effect of CRH, thereby increasing ATCH secretion\textsuperscript{29-31}</td>
</tr>
<tr>
<td></td>
<td>Pancreatic islet cells</td>
<td>Decreasing blood glucose levels via induction of insulin secretion\textsuperscript{32,33}</td>
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</table>
Measurement of vasopressin by a surrogate marker

Interestingly, most insights concerning the spectrum of functions of vasopressin have been gained despite the fact that studying vasopressin in the clinical situation is difficult. Some authors attribute this to imprecision of the radioimmunoassay that is used to measure vasopressin.34 Though actually this assay is quite reliable when executed correctly.35,36 Nevertheless, the vasopressin assay is time-consuming and technically difficult and therefore prone to errors. An even more important issue might be sample handling during the pre-analytical phase, when blood samples are drawn and stored. Ex-vivo stability of vasopressin is limited, thus introducing a significant bias.35,37,38

To resolve these issues, copeptin was introduced as surrogate marker for vasopressin.39 Copeptin is a 39-aminoacids large glycopeptide.40 Copeptin and vasopressin are split from a mutual precursor protein in equimolar amounts, which is the principal rationale for using plasma copeptin levels as reflection of vasopressin secretion. The physiologic function of copeptin itself is not yet ascertained. It was suggested that it assists in the correct protein folding of vasopressin.41 Use of copeptin as surrogate marker is supported by measurements of plasma levels of both peptides in various clinical situations. Copeptin and vasopressin have been shown to correlate well in healthy subjects and in various patients populations.39,42-44 Due to its larger size, copeptin can be measured by a semi-automatic assay, which is easy to run and only takes around 2 hours, in contrast to the 48 hours of the vasopressin assay. Moreover, copeptin is believed to be more stable during cold storage. Therefore, copeptin provides an interesting possibility when it comes to study vasopressin on a large scale.

Vasopressin and copeptin in kidney disease

Despite the fact that vasopressin is evidently important to the functionality of the kidneys, it has also been linked to mechanisms promoting the progression of chronic kidney disease.45,46 In both experimental studies and in short-term clinical studies is shown that infusion of vasopressin results in an increased renal blood flow, glomerular hyperfiltration, proteinuria, and glomerulosclerosis.45,47,50 In addition, several studies demonstrate an association of plasma copeptin and disease progression in various populations, namely renal transplant recipients51, patients with diabetes52,54, patients with microalbuminuria55 and in patients with autosomal dominant polycystic kidney disease (ADPKD).56,57 The association of a higher copeptin with a faster decline in kidney function is seen as further evidence for the detrimental effect of vasopressin. However, it is wise to interpret these data with caution. As protein of small molecular size, copeptin could in theory be subjected to renal clearance as primary route of elimination. Consequently, its plasma levels could rise in concurrence with decline of glomerular filtration rate. The strength of the association between copeptin and the progression of kidney disease found in epidemiologic studies may therefore only partly represent vasopressin activity. It may also be explained as a reflection of a lowered clearance of copeptin, and therefore of
kidney function per se. Before copeptin measurement can replace direct measurement of vasopressin, clarification of the correct interpretation of copeptin plasma levels in the context of kidney disease is required.

Vasopressin is studied in the context of various kidney diseases. Though there is one disease in particular, in which the study of vasopressin has gained attention, namely in ADPKD. This is a genetic condition in which the ongoing development and growth of cysts impair kidney function.\textsuperscript{58} On top of the abovementioned mechanisms that are universally detrimental in all kidney diseases, vasopressin affects the pathophysiology of ADPKD directly: binding of vasopressin to its V2 receptor accelerates cyst formation. This role of vasopressin in ADPKD disease pathophysiology was first demonstrated in an extensive number of preclinical studies. Thereafter, clinical trials have shown a beneficial effect of a vasopressin V2 receptor antagonist on disease progression. For the interested reader, more information regarding the clinical manifestations, pathophysiology and management of ADPKD is presented in Chapter 2 of this thesis. As result of the iatrogenic nephrogenic diabetes insipidus induced by the use of this V2 receptor antagonist, plasma vasopressin levels increase considerably. Tolvaptan is highly selective for the V2 receptor, and does not block vasopressin action on other types of vasopressin receptors, the V1 and V3 receptors.\textsuperscript{59} In this context, these effects of vasopressin can be studied in detail.

**Aim of this thesis**

The general aim of this thesis is to improve interpretation of copeptin as marker of vasopressin and use these insights to study the various effects of vasopressin throughout the body.
Outline of this thesis

To start, the first part of this thesis addresses the correct measurement and interpretation of copeptin as marker for vasopressin. Chapter 3 describes the effects of various sample handling conditions on copeptin concentration, such as centrifugation force and cold storage, and compared these with the effects of these conditions on vasopressin concentration. Thereafter, in Chapter 4, a comparison between plasma copeptin and vasopressin concentrations is made in a cohort of patients with a varying degree of kidney impairment. Thereafter, the final chapter of the first part of this thesis, Chapter 5, was designed to further elucidate the clearance mechanism of copeptin, to provide insight on the correct interpretation in the context of renal disease. The first part of this thesis is therefore entitled ‘Measurement of copeptin as marker for vasopressin’.

The second part of this thesis describes how measurement of plasma vasopressin concentration and assessment of the concentration capacity of the kidneys can be of help in diagnosing disease and estimating disease severity. Chapter 6 is a case study, in which measurement of copeptin gave more certainty regarding the cause of an acute hyponatremia. Chapter 7 is a commentary on the correct interpretation of copeptin levels in when measured in search for the underlying cause of the polyuria-polydipsia syndrome. The final chapter of this part, Chapter 8, investigates whether the concentrating capacity, represented by the urine-to-plasma urea ratio, might be a good marker for rate of future kidney function decline in patients with autosomal dominant polycystic kidney disease. The concentrating capacity of the kidney is dependent on the antidiuretic effect of vasopressin together with a proper build-up of an osmolar gradient. The second part of this thesis is entitled ‘The antidiuretic effect of vasopressin’.

The third part of this thesis focuses on other effects of vasopressin. These effects were studied in the context of treatment with a selective V2 antagonist, tolvaptan. Tolvaptan is prescribed to patients with ADPKD to halt disease progression. This drug impedes vasopressin to exert its antidiuretic effect, thereby causing a state of nephrogenic diabetes insipidus. This results in renal water loss, in an increased plasma osmolality and in an increased release of vasopressin from the pituitary. These elevated vasopressin concentrations could cause various off-target effects, via activation of the V1 and V3 receptors. The effect on plasma glucocorticoids, on acid-base homeostasis and on blood pressure are described in Chapters 9, 10 and 11 respectively. The third part of this thesis is therefore entitled ‘Other effects of vasopressin’.

Finally, in Chapter 12, the outcomes and conclusions of the previous chapters are summarized and discussed. This chapter concludes with recommendations for future studies to continue this line of research.
References


