Vasopressin in chronic kidney disease, in particular ADPKD

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General discussion
Summary

Arginine vasopressin (AVP) is an important hormone for water homeostasis of the body. Vasopressin is released from the pituitary gland upon an increase in plasma osmolality or a decrease in blood volume and blood pressure.\textsuperscript{1,2} Vasopressin facilitates water reabsorption in the collecting ducts of the kidney, leading to concentration of the urine in order to restore water balance. Importantly, also detrimental effects of vasopressin on the kidney have been described as well. It is suggested that vasopressin influences the microcirculation of the kidney and thereby induces hyperfiltration, proteinuria, glomerulosclerosis and renal function loss in the long run.\textsuperscript{3,4} Moreover, in autosomal dominant polycystic kidney disease (ADPKD) specific deleterious effects of vasopressin have been found.

ADPKD is the most common hereditary kidney disease and is characterized by progressive cyst formation in the kidneys leading to end-stage renal failure in 70\% of patients usually between the age of 40 and 70 years. In the Netherlands approximately 6,500 people have ADPKD, of whom 1,500 are dependent on renal replacement therapy.\textsuperscript{5} The pathophysiology of ADPKD is not yet fully understood, but research conducted in the last decade acknowledges an important role for vasopressin in cyst growth and consequently disease progression.\textsuperscript{6} Vasopressin stimulates production of cyclic adenosine monophosphate (cAMP) by adenylyl cyclase (AC) at the basolateral side of the collecting ducts of the kidney. cAMP is needed to facilitate migration of aquaporine-2 channels into the apical cell membrane in order to reabsorb water, but also stimulates proliferation of tubular cells and chloride driven fluid secretion. These latter effects contribute to cyst growth and consequently disease progression.\textsuperscript{6} The concentration range of circulating vasopressin in chronic kidney disease patients, and specifically in ADPKD patients, is not clear. Neither has it been investigated whether vasopressin levels differ in ADPKD patients when compared to other chronic kidney disease patient groups.

In this thesis, vasopressin and its surrogate marker copeptin were studied in chronic kidney disease and in particular in ADPKD. The central aim was to study to what extent vasopressin levels are elevated, and whether elevated vasopressin levels may be a causal factor in disease progression. This thesis is subdivided into three parts. In part 1 copeptin is validated as a surrogate marker for vasopressin, in part 2 the physiological role of vasopressin is investigated in chronic kidney disease and ADPKD and, finally, in part 3 vasopressin is investigated as possible causal factor in chronic kidney disease progression.
Part 1: Copeptin as surrogate marker for vasopressin

Copeptin is part of the vasopressin precursor hormone pre-pro-vasopressin. Splicing of this precursor leads to the release of copeptin and vasopressin in equimolar amounts. It has been demonstrated that copeptin levels correlate well with vasopressin levels during physiological changes in plasma osmolality, from water excess to dehydration.

The studies described in part 1 of this thesis were performed to develop a protocol for optimal pre-analytical sample handling for measurement of copeptin and vasopressin in clinical and epidemiological studies, and to validate copeptin as marker for vasopressin in patients with impaired kidney function.

In Chapter 3 the effect of centrifugation speed, various short-term storing conditions pre- and post-centrifugation, long-term freezer storage, and repeated freeze-thaw cycles on both copeptin and vasopressin levels in blood and plasma of 10 healthy volunteers was investigated. The stability of vasopressin varied widely between the different study conditions. Especially the impact of different centrifugation speeds on vasopressin was impressive, whereas copeptin was not affected by centrifugation speed or any of the other study conditions. Because of this limited ex-vivo stability of vasopressin, the use of a strict sample handling protocol in measurements of vasopressin is advocated. Blood samples should be collected on ice and centrifuged at 4°C immediately after collection at preferably 2000 g or higher. Short-term storage of plasma samples is preferred at 4°C for a maximum time span of 24 hours or, if storage for a longer period is inevitable, plasma samples should be stored at -80°C. Repeated freeze-thaw cycles should be avoided. A far less strict protocol for copeptin is required. This makes measurement of copeptin as surrogate for vasopressin an attractive alternative, especially when samples are used that have been stored frozen during prolonged periods of time, that have undergone freeze-thaw cycles, or that are collected without a strict sample handling protocol.

In Chapter 4 and Chapter 5 we aimed to unravel whether increased copeptin levels in chronic kidney disease patients accurately reflect vasopressin levels or are the result of decreased renal clearance. In Chapter 4 we measured copeptin in 134 healthy kidney donors before and after kidney donation and in 122 ADPKD patients. In this study, we found no association between glomerular filtration rate (GFR, measured as iothalamate clearance) and copeptin levels in healthy kidney donors pre- or post-donation. Moreover, in these participants copeptin levels did not change after kidney donation, despite a significant decrease in GFR of 40%. In contrast, in ADPKD patients a significant association was found between GFR and copeptin levels. These data suggest that copeptin is more than merely a filtration marker and that copeptin is associated with disease severity in ADPKD.

In Chapter 5 we investigated the association between renal function and the copeptin/vasopressin ratio in 16 healthy volunteers and 114 chronic kidney disease patients with a wide range of renal function. We found levels of copeptin and vasopressin to be elevated...
in patients with impaired renal function when compared to healthy controls. Because both analytes were increased to the same extent, the copeptin/vasopressin ratio was independent of renal function in these subjects. However, in patients with a glomerular filtration rate (eGFR) below 30 ml/min/1.73m², copeptin increased to a greater extent than vasopressin. The results of Chapters 4 and 5 suggest that copeptin is a reliable marker for vasopressin, even in patients with impaired renal function, at least when eGFR is above 30 ml/min/1.73m². In literature a role for renal clearance in the metabolism of copeptin has been suggested as several studies found an inverse association between copeptin and renal function.10-15 Our results show, however, that these elevated copeptin levels seem to be an accurate reflection of vasopressin levels and are not the effect of reduced renal clearance. Nonetheless, the marked increase in copeptin in severe renal insufficiency does imply a modest renal component in the total clearance of copeptin. In clinical and epidemiological studies in which patients are included with an eGFR less than 30 ml/min/1.73m², it is therefore advised to adjust associations of copeptin levels with markers of disease severity or progression for kidney function.

Part 2: Urine concentrating capacity and vasopressin in chronic kidney disease

The first studies examining the maximal urine concentrating capacity in patients with chronic kidney disease were published around sixty years ago. These studies found an impaired ability to concentrate urine in patients with decreased kidney function.16-19 Two major renal causes were described that could possibly underlie this impairment: a decrease in functioning nephrons and a pathological process in the residual nephrons.17,20,21 Patients with ADPKD seem to have a specific defect in the ability to concentrate urine. The mechanism that causes this concentrating defect is not exactly known. Martinez-Maldonado et al. found an impaired urine concentrating capacity in ADPKD patients, even in patients with an intact eGFR, implying a defect of the tubular function with a sufficient functioning glomerular filtration system.22 Both an impaired medullary osmotic gradient due to cysts induced abnormal interstitial architecture and an insensitivity to vasopressin e.g. due to a receptor defect have been suggested to cause this tubular dysfunction in ADPKD.23-25 Theoretically, a decrease in renal concentrating capacity could also have a central cause, i.e. impaired vasopressin release by the pituitary gland. A reliable assay for vasopressin was not available at the time when these studies were performed and therefore vasopressin was not measured in these studies. A definitive conclusion upon a potential central vasopressin deficit in ADPKD can therefore not be drawn. Part 2 of this thesis focused on increasing the current knowledge of the maximal urine concentrating capacity and vasopressin response in chronic kidney disease, in particular in ADPKD.
In Chapter 6 water deprivation tests were performed in 15 ADPKD patients in early stage of their disease, as reflected by a normal eGFR, and 15 healthy volunteers matched for age, sex and eGFR, to determine the maximal urine concentrating capacity and vasopressin and copeptin response. The maximal urine concentrating capacity was lower in ADPKD patients, with particularly a lower maximal achieved urine urea concentration, than in healthy subjects. After reaching maximal urine concentrating capacity, plasma osmolality, vasopressin and copeptin levels were significantly higher in ADPKD patients in comparison to healthy volunteers. To investigate whether this concentration deficit is amplified in ADPKD patients with later stage disease, in Chapter 7 similar water deprivation tests were performed in ADPKD patients with impaired kidney function, and compared with non-ADPKD patients with a similar level of impaired kidney function. IgA nephropathy patients were chosen as control group, matched for age, sex, and eGFR, to compare the concentrating capacity and vasopressin response in patients with predominantly tubulo-interstitial damage (ADPKD) to patients with predominantly glomerular dysfunction (IgA nephropathy). This study showed that ADPKD patients had a more severely impaired urine concentrating capacity with, again, a lower maximal achieved urine urea concentration in comparison to IgA nephropathy patients. Both study groups, however, had similar elevated vasopressin and copeptin levels at baseline and during water deprivation. Furthermore, more severe ADPKD, assessed as a higher total kidney volume, was positively associated with plasma osmolality, copeptin and albuminuria, and with a more severely impaired urine concentrating capacity during water deprivation. The results of both water deprivation studies indicate that the impairment in the ability to concentrate urine in patients with chronic kidney disease most likely is not based on a central deficiency to secrete vasopressin levels as the vasopressin levels that were found in these studies were high. The fact that a urine concentrating impairment was found in ADPKD patients with still a normal kidney function, and that this was based on especially an impaired urine urea concentration, suggests a role for interstitial damage in the kidney. We hypothesize that destruction of the medullary architecture due to the development of cysts leads to disruption of urea transporters and the intra-renal urea recycling in the medulla. This in turn leads to a decrease in urea excretion and urine concentrating ability. In IgA nephropathy patients an impairment of the urine concentrating capacity was seen as well, although less profound and with a higher maximal urine urea concentrating capacity in comparison to ADPKD patients with similar eGFR. This shows that etiology of the kidney disease is of importance to determine the extent and origin of the urine concentrating impairment. It should be emphasized that in ADPKD as well as in IgA nephropathy patients circulating vasopressin levels during normal conditions and during water deprivation were elevated in comparison to healthy controls. Given the possible deleterious effects of vasopressin on the kidney, this observation is of importance and further investigated in part 3.
Part 3: Vasopressin as causal factor in chronic kidney disease progression

Elevated vasopressin levels found in part 2 of this thesis are of importance as detrimental effects of vasopressin on kidney disease progression have been described. In this thesis it was hypothesized that when kidney damage occurs, urine concentrating capacity declines, leading to an increase in vasopressin, which in turn causes kidney damage. Thus a vicious circle is created that predisposes for progressive kidney function loss. The third part of this thesis aims to investigate whether vasopressin is indeed a causal factor in chronic kidney disease progression in general and in ADPKD in particular, and how patients that may benefit from treatment by vasopressin blockade can be identified.

In Chapter 8 the association of copeptin with disease severity and progression was investigated in a prospective cohort of 59 IgA nephropathy patients. In this study strong associations were found between copeptin and various markers of kidney damage. Baseline copeptin was significantly associated with incidence of the composite renal outcome and its individual components doubling of serum creatinine, end stage renal failure and start of immunosuppressive therapy during 5 year follow-up. When investigating copeptin in multivariate Cox regression analyses, copeptin added prognostic value over blood pressure, proteinuria and eGFR. These results can be added to a growing body of evidence supporting the independent prognostic role of copeptin in chronic kidney disease. Our research group has previously shown that copeptin is associated with prevalence of microalbuminuria in a large population-based cohort and has predictive value with respect to renal outcome in several populations, including renal transplant recipients and patients with type 2 diabetes. These studies all support the hypothesis that vasopressin has a pathophysiological role in the progression of chronic kidney disease.

Regarding ADPKD, vasopressin may have a specific additional detrimental role, and the body of evidence supporting this role is growing since the last decade. Strangely enough, studies investigating whether in ADPKD vasopressin is associated with disease progression are lacking. This might be caused by the fact that vasopressin is difficult to measure. Using copeptin as surrogate (as described in part 1 of this thesis) made it possible to investigate this question. In Chapter 9, copeptin was investigated in 79 ADPKD subjects as prognostic marker for renal function decline. In these patients, renal function was assessed during short-term follow-up (3 years) by inulin clearance and during long-term follow-up (11 years) by creatinine based estimated GFR. Baseline copeptin concentration was inversely associated with change in mGFR during short-term follow-up, as well as with change in eGFR during long-term follow-up. These associations were independent of age, gender and baseline kidney function (mGFR and eGFR, respectively). The findings of this study are in line with intervention studies. Treatment with a vasopressin V2 receptor antagonist delayed disease progression in animal models
for polycystic kidney disease. A recent multicenter randomized controlled trial corroborated in patients with relatively early stage disease found that the vasopressin V2 receptor antagonist tolvaptan slowed the increase in total kidney volume and the decline in kidney function over a 3 year period. Although a break-through in ADPKD treatment, optimal timing and dosage of the drug are yet debated. All experimental as well as clinical studies investigated a fixed dose treatment regimen in relatively early stage of disease. It has been suggested that disease severity and treatment duration may influence treatment efficacy, as indicated by a decline in aquaretic response to V2 receptor antagonism in later stage disease and during prolonged treatment. Therefore, in Chapter 10 optimal time of start and optimal dosage of V2 receptor antagonist treatment was examined in a PKD mouse model. The V2 receptor antagonist was administered to Pkd1-deletion mice in a fixed dose or in a titrated dose, based on water intake. Treatment was started early or late (21 or 42 days postnatal). In this study, water intake was significantly higher throughout the experiment in the titrated dose treatment group compared to the fixed dose treatment group. Early initiated fixed dose treatment reduced total kidney weight and cyst ratio, but lost its renoprotective effect later during the experiment. In contrast, titrated dose treatment was able to maintain the renoprotective effect. Titrated dose treatment, however, was also associated with a high early termination rate in comparison with fixed dose treatment. Late start of treatment, fixed or titrated dose treatment, did not show any renoprotective effect at all. Translating experimental data into clinical practice is difficult and should be done with caution. This study shows that a better therapeutic response can be achieved when higher doses of the V2 receptor antagonist are used. This suggests that dose titration of a V2 receptor antagonist in clinical practice, when disease progresses and the aquaretic response to the drug decreases, may be beneficial. However, this renoprotective effect was counteracted by significant side effects. Moreover, this rigorous treatment regimen did not overcome therapeutic unresponsiveness when started in a later stage of the disease. Combination therapy, for instance with a somatostatin analogue, may allow to use lower doses of each single drug, thereby limiting adverse events, while optimizing renoprotective efficacy.

At present it is advocated to use urine osmolality as measure for the degree of suppression of vasopressin. As such urine osmolality could be used to select patients with assumed high vasopressin levels for treatment by vasopressin reduction, either by increasing fluid intake or by prescribing a vasopressin V2 receptor antagonist. In healthy subjects with normal kidney function, vasopressin levels indeed correlate positively with urine osmolality. In line, it has been suggested that in ADPKD patients a urine osmolality under 285 mOsmol/l (i.e., a urine osmolality below plasma osmolality) indicates a water intake appropriate to suppress vasopressin levels. However, the impaired urine concentrating capacity in ADPKD patients found in part 2 of this thesis raised concerns regarding the usefulness of urine osmolality as marker for vasopressin
activity in this specific patient population. In Chapter 11, we therefore investigated whether urine osmolality and urine to plasma osmolality ratio are accurate markers for circulating vasopressin levels. The findings in this chapter show that in ADPKD patients both urine osmolality and urine to plasma osmolality ratio are not appropriate to monitor vasopressin levels, measured as plasma copeptin concentration. Patients with advanced disease showed low urine osmolality, but high copeptin levels. Most likely, in these patients a low urine osmolality does not reflect adequate suppression of vasopressin, but merely that these patients have an impaired urine concentrating capacity leading to a decrease in urine osmolality as well as an increase in plasma osmolality and an increase in vasopressin levels. Our data suggest that plasma and urine osmolality cannot be used to identify ADPKD patients with a high copeptin (i.e. vasopressin) concentration that are at risk for a more rapid rate of kidney function decline during follow-up. For this purpose measuring copeptin concentration may be a better alternative.

Future perspectives

Part 1
Although the results of part 1 of this thesis show that copeptin is mostly cleared extra-renally, the exact metabolic fate of copeptin is still unknown. The marked increase in copeptin in severe renal insufficiency seen in Chapter 5 does imply at least a modest renal component in the total body clearance of copeptin. Experimental studies that determine renal clearance, half-life, and tissue deposition of copeptin would greatly enhance acceptance of copeptin as a valid marker in epidemiologic studies in kidney disease by the scientific community, especially for research in subjects with a GFR <30 ml/min/1.73m². Such studies have yet to be performed.

Part 2
The finding of an impaired urine concentrating capacity and subsequently high vasopressin levels in both ADPKD and IgA nephropathy patients with impaired kidney function described in part 2 of this thesis suggest these findings may be applicable to chronic kidney disease in general as ADPKD and IgA nephropathy are two very different renal diseases in terms of etiology. However, before this can be concluded, water deprivation tests and measurement of vasopressin should also be performed in patients with chronic kidney disease other than IgA nephropathy and ADPKD. It will be of interest to establish whether patients with chronic kidney disease have an impaired urine concentrating capacity and consequently elevated vasopressin levels, especially when this will be the case already in early stage of disease. Already in the sixties it was shown in patients with hypertension but normal serum creatinine levels that the level of urine concentration in early morning urine samples detects patients with early stage
renal disease. Tibblin and Vikgren, the authors of this study, advocated to determine the urine concentrating capacity as a relatively simple, non-invasive marker for renal disease in subjects of the general population. Whether the urine concentration of an early morning urine sample can indeed select subjects with an impaired urine concentrating capacity and detect renal disease in an early stage should be further investigated. Such a procedure could identify patients at risk for renal disease progression, that may benefit from early start of treatment.

**Part 3**

The outcomes of part 3 of this thesis, i.e. that copeptin independently predicts renal function loss in IgA nephropathy as well as ADPKD patients, are in line with the described deleterious effects of vasopressin on the kidney. In order to decrease vasopressin levels, sufficient hydration could be of importance. In chronic kidney disease the medicinal use of water to decrease vasopressin levels has been described several times. In 5/6 nephrectomized rats an increase in water intake was protective with a less increase in urine protein excretion and systolic blood pressure during follow-up as in rats with normal water intake. Recently, two community-based studies investigated the association between water intake and kidney function. In a cross-sectional study the group with a high self-reported mean daily fluid intake had a lower prevalence of chronic kidney disease. In the other study with a prospective design, study participants with the highest 24 hour urine volume at baseline showed the lowest rates of decline in eGFR thereafter. These studies suggest that progressive kidney damage can be delayed by sufficient hydration. However, large scale randomized controlled trials are needed to test this hypothesis. One such study, the Water Intake Trial (WIT), is ongoing.

As shown in the TEMPO 3:4 trial V2 receptor antagonist therapy attenuates disease progression in ADPKD. The findings in Chapter 10 suggest that treatment response can be optimised by up titration of the dose. However, as also shown in this chapter, adverse effects may limit the widespread clinical use of high dose V2 receptor antagonists. Adverse effects include thirst, polydipsia, polyuria, and nycturia, which can cause sleep disturbance. These side effects, in combination with the variable disease course in ADPKD, prompt the need for identification of patients with a high likelihood of rapid disease progression that are most likely to benefit from treatment, because in such subjects the benefit to risk ratio will be higher. This thesis suggests that copeptin could be such a marker, but future research is needed to confirm this before copeptin measurement can be used in clinical practice. In addition, even the rigorous treatment regimen in Chapter 10 did not overcome therapeutic unresponsiveness when started in a later stage of the disease. To enhance therapeutic efficacy, combination therapy could be of more use. A number of drugs are under investigation, of which somatostatin analogues perhaps seem most promising, because they induce a similar effect on intracellular
cAMP production as V2 receptor antagonists. Both drug classes inhibit adenylyl cyclase, the enzyme that converts adenosine triphosphate (ATP) into cAMP. Interestingly, Hopp et al recently showed in a Pkd1 experimental model that combination treatment with a somatostatin analogue and a V2 receptor antagonist led to lower cAMP levels and less cyst progression when compared to monotherapy with either of both drugs. These experimental data indicate that in ADPKD patients a combination of these drugs may allow to use lower doses of each single drug, thereby limiting the incidence and severity of adverse events, while retaining renoprotective efficacy. These experimental results are promising and, given the results of Chapter 10, seem a more logical direction for the design of future clinical trials than testing the renoprotective efficacy of even higher doses monotherapy than those that are used at present.

Vasopressin, causal factor or innocent bystander in chronic kidney disease?

The work presented in this thesis brings us closer to understanding the role of vasopressin in chronic kidney disease. It is important to realize that circulating vasopressin levels increase in patients with kidney damage, especially when interstitial damage occurs as this leads to impairment of urine concentrating ability. The dramatic progression of interstitial damage seen in ADPKD patients due to cyst growth is a classic example of this mechanism. However, also in other types of chronic kidney disease this impairment in urine concentrating capacity and subsequently elevated vasopressin levels can be observed, for instance in IgA nephropathy as shown in this thesis. In ADPKD, substantial evidence has emerged regarding the effect of vasopressin on cyst growth and kidney function deterioration. In the last couple of years, several study groups across the world concluded that vasopressin is a causal factor involved with ADPKD progression. The results obtained with vasopressin V2 receptor blockade in mice with polycystic kidney disease (Chapter 10) are in agreement with this conclusion. Using copeptin as marker for risk for rapid disease progression in ADPKD seems therefore promising. The fact that copeptin also predicts disease progression in IgA nephropathy, suggests that vasopressin is more than just an innocent bystander in this disease as well, and might be causally related. However, the evidence for this hypothesis presented in this thesis is indirect and further research is needed, in IgA nephropathy in particular, but also in chronic kidney disease in general. A randomized controlled trial in which vasopressin activity is blocked will provide the evidence that is needed. Increasing water intake to suppress plasma vasopressin concentration may be the preferred option in a chronic kidney disease population. Whether such a dietary intervention is feasible on the long-term, however, remains to be proven. If not, treatment with a vasopressin V2 receptor antagonist can be studied.
References


