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TABLE OF CONTENTS

Abbreviations and symbols

C_{H_2O}	Free water clearance
$T^{C}{}_{H_2O}$	Negative free water clearance
C_{osm}	Osmolal clearance
A_1	Adenosine 1 receptor
A_2	Adenosine 2 receptor
ACE	Angiotensin converting enzyme
AQP	Aquaporin
AVP	Arginine vasopressin
BSA	Bovine serum albumin fraction V
ClC	Chloride channel
COX	Cyclooxygenase
DMPX	1,3-dipropyl-7-methyl-8-(3,4-dimethoxystyryl)xanthine
DPCPX	1,3-dipropyl-8-cyclopentylxanthine
EnaC	Epithelial Na^+ channel
FE	Fractional excretion
FF	Filtration fraction
GFR	Glomerular Filtration Rate
glut	Glucose carrier protein
GTB	Glomerulotubular balance
KCCl	Potassium chloride cotransporter
L-NAME	N^{ω}-nitro-L-arginine methyl ester
NCC	Sodium chloride cotransporter
NHE3	Sodium-proton exchanger type 3
NKCC2	Na, K, 2 Cl cotransporter
NO	Nitric Oxide
NOS	Nitric oxide synthase
P_f	Osmotic water permeability
RBF	Renal Blood Flow
RPF	Renal perfusate flow, renal plasma flow
RPP	Renal perfusion pressure
SGLT	Sodium glucose cotransporter
TAL	Thick ascending limb of Henle's loop
TGF	Tubuloglomerular Feedback
TTKG	Transtubular potassium gradient
UT	Urea transporter
Uv	Urine flow

List of Tables

List of Figures

Chapter 1: General Introduction

General Introduction

This introduction will first deal with aspects of renal function that will be more explicitly investigated in the isolated perfused rabbit kidney and secondly an overview will be given of the isolated perfused kidney preparations.

1.1 The kidney function and important influence on homeostasis

All cells of the body are bathed in the interstitial fluid. This fluid is the environment in which these cells must develop their activities of metabolism and growth. Obviously, the composition and volume of this fluid compartment must remain constant within narrow limits. Abnormal volumes of vascular and interstitial fluids impair cardiovascular function and an abnormal composition of interstitial fluid will impair cellular function. Constancy of these compartments is called 'homeostasis', which is defined as the maintenance of constant conditions in the internal environment (Hall and Guyton, 2000).

The kidneys have a very important task, for slight changes in fluid, electrolyte or nitrogen balance might lead to disturbing changes in the composition and volume of the interstitial as well as the intracellular fluids. In fact the intracellular fluid composition is largely dependent on the composition of the extracellular/interstitial fluid. Accumulation of urea, a nitrogenous waste product, can disturb neuron and brain function. Insufficient excretion of sodium and water can lead to an increase of extracellular volume, that can eventually result in hypertension. Failure of the kidneys to control the balance of potassium might lead to hyper- or hypokalemia, with disastrous consequences for excitable tissues: neurons and muscles, including the heart. Finally in the case of inability to control the output of H^+, acidaemia or alkalaemia may result in deadly situations (Rose, 1994; Rose and Post, 2001).

Consequently the kidneys play an essential role in homeostasis and therefore acute failure of renal function may lead to death within 48 hours of onset, while the survival over a period of three months may also show a high rate of morbidity and mortality (Frost et al., 1993; Roubicek et al., 2000; Naqvi et al., 2003; O'Hare et al., 2003).

Moreover, the kidneys are also widely involved in metabolism and elimination of pharmacological agents or their metabolites. Renal elimination of aspirin, a widely used drug, is well known. Not only do the kidneys control excretion of drugs or their metabolites, but renal function is often affected by the drugs and in some cases toxicity might occur.

Nephrotoxic effects of gentamycin, a widely used antibiotic is generally known. In some instances, the excretion of the drug by the kidney is necessary in order to be effective. For example, antibiotics that must be able to reach the bladder cavity in case of cystitis must be excreted in order to reach therapeutic levels in the bladder. In fact, the primary route of drug or metabolite elimination in the mammalian system is the kidney (Bekersky, 1983a; Bekersky, 1983b).

Renal function or dysfunction can therefore affect the interstitial fluid and may influence the function of other organs and organ systems in health, disease and therapeutic situations. The opposite is also true, and renal function is largely influenced by the (dys)function of other organ(system)s. Diabetes mellitus, cardiac failure and hepatic cirrhosis are just a few examples of disease states affecting renal function. Besides, the kidneys are also under influence of nervous (norepinephrine) and endocrinological factors (AVP, aldosterone) and part of the renal function is also an endocrinological one (renin, erythropoietin, vitamin D_3 or cholecalciferol) (Hall and Guyton, 2000).

1.1.1 Hemodynamic properties of the kidney

1.1.1.1 Structure of the renal circulation

Structurally and morphologically the rabbit kidney has a lot in common with human kidneys. The ratio of juxtamedullary and cortical nephrons and maximal concentrating ability are some factors that seem to be similar. Rat kidneys differ somewhat more in anatomy and structure from mammalian kidneys like those of rabbits and humans. Therefore one might expect a comparable function in human and rabbit kidneys, hence exploration of renal function in the rabbit could improve our understanding of the function of the human kidney (Bankir and De Rouffignac, 1985). On the other hand it has been demonstrated that there are differences at the molecular level along segments of the nephron, which may lead to a slight variation in function (Loffing and Kaissling, 2003).

Renal function depends largely on the blood supply and vascular structure of the kidneys, receiving about 20 % of the cardiac output. Hence systemic hemodynamic changes are likely to influence renal blood flow and function (Hall and Guyton, 2000; Dworkin et al., 2000).

In this context it is worthwhile mentioning that renal vasculature has a special structure and is uniquely adapted to accommodate the complex and sometimes conflicting requirements of supplying nutrients and removing tubular reabsorbate.

Primarily plasma must be filtered in the glomerulus. This glomerulus is a vascular structure through which large amounts of fluid are filtered from the blood and further processed in the tubular structure of the nephron, the smallest functional unit of the kidney. In mammals it is a complex microvascular structure composed of endothelial, epithelial, and mesangial elements. The coordinated actions of these cells and the physical forces acting on them make the separation possible of an ultrafiltrate from the large volume of plasma that normally flows through this capillary network. The glomerulus consists of a network of branching capillaries that, contrary to the major part of capillaries in the body, have a high hydrostatic pressure. This glomerular capillary network is unusual in that it is arranged in series between upstream and downstream resistance vessels and it is this arrangement that facilitates precise regulation of glomerular capillary hydraulic pressure and plasma flow rate by selective alterations in afferent and/or efferent arterioles (Dworkin and Brenner, 2000; Maddox and Brenner, 2000).
The total glomerulus is encapsulated in the capsule of Bowman, and the fluid that is filtered from the glomerular capillaries flows into this structure to enter the first part of the tubular system i.e. the proximal tubule (Hall and Guyton, 2000).

The glomerulus receives its blood from the afferent arteriole and the glomerular capillaries coalesce at the end to form the efferent arteriole. The structure of efferent arterioles varies as with cortical location. Superficial (cortical) efferent arterioles mostly form a second network of capillaries, the peritubular capillaries. In contrast to the efferent arterioles of superficial glomeruli, those of juxtamedullary glomeruli most frequently cross the corticomedullary junction to enter the outer stripe of the outer medulla where they give rise to descending vasa recta (Hall and Guyton, 2000).
Single nephron glomerular filtration rate and size of glomerulus in juxtamedullary nephrons in rabbit kidneys appear to be greater compared to the superficially located cortical nephrons, a phenomenon that has been observed in other species as well (Bankir and Rouffignac, 1976).
The efferent flow from the glomeruli is directed to the peritubular capillary plexus in the cortex or to the descending vasa recta in the outer medulla. Recovery of tubular reabsorbate is accomplished by uptake across the walls of highly permeable, fenestrated capillaries in the cortex and ascending vasa recta in the medulla (Pallone, 2000).

1.1.1.2 Autoregulation of the renal blood flow and glomerular filtration rate

The glomerular filtration rate (GFR) is precisely regulated and remains relatively constant despite marked changes in the volume status and systemic hemodynamics. Overall, renal blood flow varies very little despite changes in renal perfusion pressure within the range of 80 to 140 mmHg, a phenomenon called "autoregulation". Autoregulation is observed in both denervated and isolated kidney preparations and therefore appears to be a property intrinsic to the kidney and independent of extrarenal or systemic hemodynamic events (Hall and Guyton, 2000; Maddox and Brenner, 2000).

Variation in arterial blood pressure causes variation in glomerular capillary pressure and a tendency for the GFR to vary. The kidneys are fully equipped to react on variation in arterial blood pressure and are able to keep a relatively constant blood flow within a certain range of perfusion pressure variations (Holstein-Rathlou and Marsh, 1994).

It is worthwhile to mention that this phenomenon has also been demonstrated in studies performed on rabbits (Ott and Vari, 1979).

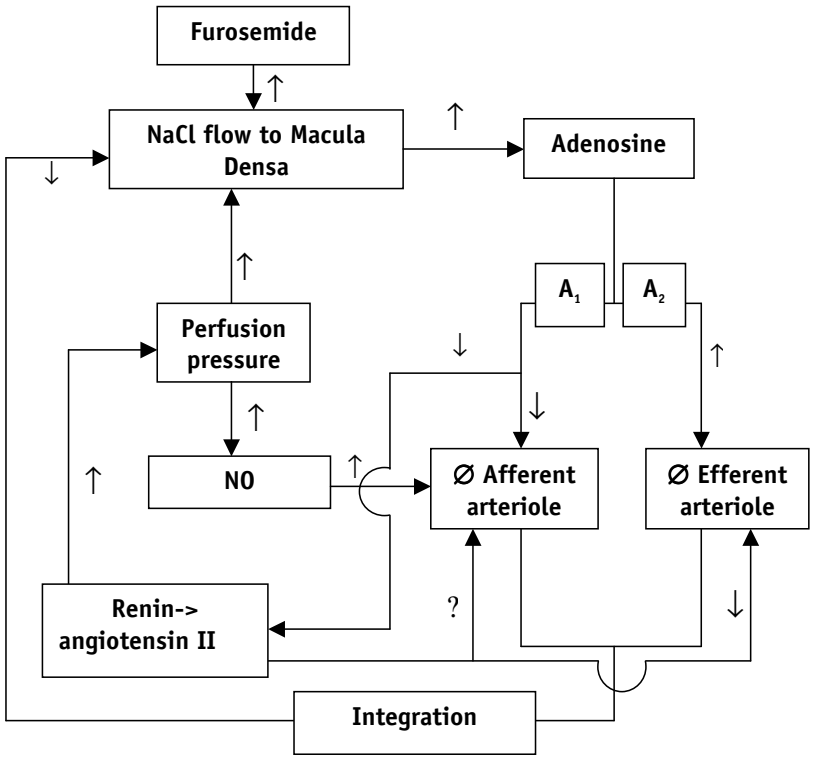

Figure 1.1: Tubuloglomerular feedback. Ø, vessel size.

The process of tubuloglomerular feedback (figure 1.1) plays a role in autoregulation of both renal blood flow and glomerular filtration rate. The tubuloglomerular system is a mechanism that operates in each nephron to stabilize nephron blood flow, single nephron GFR and tubular flow rate. It transfers information derived from the flow rate of tubular fluid to the glomerulus (Holstein-Rathlou and Marsh, 1994). The sensed variable is probably the NaCl concentration or flow of tubular fluid at the point in the thick ascending limb of Henle's loop in contact with the glomerular mesangium and capillary bed (macula densa) (Bell et al., 1978b; Briggs and Schnermann, 1996). Although it seems that intratubular chloride concentration plays an important role in the sensing of the macula densa, other studies have shown that fluid osmolality, sodium and potassium might as well be considered and that chloride may not be the only signal (Navar et al., 1978; Bell et al., 1978b; Bell et al., 1981a; Vallon et al., 1997; Vallon et al., 1998). A high osmolality of the renal medullary interstitium raises tubular concentrations at the bend of Henle's loop, and membrane transport of sodium chloride in the entire ascending limb of Henle reduces it to below interstitial concentrations. The higher the flow rate, the higher the mass flow of NaCl in thick ascending limbs, and the less effectively can active transport lower the concentration. The apical membranes of macula densa cells contain the NaK2Cl cotransport mechanism. Under physiological conditions, the rate of transport is dependent primarily on the Cl^- concentration in the tubular fluid (Schnermann et al., 1981). An increase in the tubular Cl^- concentration induces a change in the rate of ion transport across the apical membrane of the macula densa cells. This then generates a signal that is transmitted to the vascular smooth muscle cells of the afferent arteriole to cause vasoconstriction (Holstein-Rathlou and Marsh, 1994), but it has also been shown that the efferent arteriole might as well play a role in the tubuloglomerular feedback (TGF) mechanism (Ren et al., 2001). All studies agreed that the tubuloglomerular feedback system constitutes a potentially powerful mechanism to prevent excess losses of fluid and electrolytes by reducing GFR in settings in which distal delivery is enhanced (Dworkin and Brenner, 2000; Dworkin et al., 2000; Maddox and Brenner, 2000; Schnermann, 2002; Vallon, 2003).

The communication between macula densa and glomerular vasculature remains a point of discussion. Different mediators have been proposed, of which the first was angiotensin II via the renin angiotensin system. Recent studies however, favour more a role for adenosine, making the role for renin a more regulatory one. Prostaglandins and nitric oxide may also

influence the TGF (Itoh et al., 1985; Schnermann, 1988; Osswald et al., 1997; Schnermann, 1998; Navar, 1998). Insights from gene-targeted mice have led to the concept that adenosine mediates the TGF probably via A_1 receptors and that nitric oxide and angiotensin II play only a modulating role (Vallon, 2003). Other authors are also suggesting a role for ATP in this signalling cascade (Nishiyama and Navar, 2002; Schnermann and Levine, 2003; Bell et al., 2003).
Moreover it has been suggested that gap junctions connecting the different cellular elements of the juxtaglomerular apparatus and macula densa may also play a role in this signalling process as well as depolarisation of the macula densa cells through Cl^- exit at the basolateral side (Ren et al., 2001; Ren et al., 2002).

In spite of the previous evidence for the role of the macula densa, autoregulation of the blood flow has also been demonstrated in the absence of a macula densa or absence of an intact tubule (Maddox et al., 1974; Haberle et al., 1990a). This observation suggests that the myogenic reflex in renal blood vessels might play a major role in response to alterations in renal perfusion pressure. In fact, isolated perfused rabbit and rat afferent arterioles constrict on exposure to increased perfusion pressure i.e. stretch of vascular wall (Dworkin and Brenner, 2000). Probably this is mediated via "stretch-activated" cation channels that lead to depolarization, calcium entry, and vascular contraction (Takenaka et al., 1998; Navar, 1998). Moreover, studies have also shown that preglomerular vascular resistance is reduced with decreased renal perfusion pressure in rat kidneys with almost no functioning tubules (Steinhausen et al., 1989).
At least in rat and dog kidneys it has been demonstrated recently that the myogenic response might be the primary response and that the TGF follows secondarily (Just et al., 2001; Just and Arendshorst, 2003).

Evidence suggests that the endothelium also participates in the myogenic response in renal vessels, probably through the release of NO during shear stress. This may serve to prevent the development of a positive-feedback loop in which increased perfusion pressure causes intramural tension to increase, thereby inducing further constriction (Juncos et al., 1995).
The molecular signaling mechanisms that control vasomotor tone throughout the whole kidney are complex. Indeed, the glomerulus is the site of synthesis of and the target organ

for a large number of vasoactive humoral factors that have to interact to produce their final effect (Dworkin and Brenner, 2000; DiBona and Kopp, 2000).

1.1.1.3 Hormonal factors regulating renal circulation

The presence of adrenergic α receptors in afferent and efferent arterioles suggests that sympathetic stimulation constricts both afferent and efferent arterioles and is thereby able to decreases renal blood flow (Edwards and Trizna, 1988). Renal nerve stimulation can regulate glomerular ultrafiltration by altering vascular resistances of the afferent and efferent arterioles respectively, and angiotensin II appears to be a critical factor for the full functional expression of this stimulation (Pelayo et al., 1983; Kon and Ichikawa, 1983). On the other hand, substances like bradykinin have a more depressor activity on renal nerve activity (Niitani et al., 1988). Prostaglandins act as local modulators of both renal nerve and angiotensin II constrictive actions on glomeruli and renal microcirculation and are more involved with vasodilatory processes (Pelayo, 1988). Tonic release of nitric oxide and epoxyeicosatrienoic acids attenuates the vasoconstrictor response to angiotensin II in afferent arterioles of the rabbit (Kohagura et al., 2000). It was shown that serotonin plays a pivotal role in the suppression of autoregulation of renal blood flow by a S2-serotonergic receptor-mediated vasoconstrictor effect in the postischemic rat kidney (Verbeke et al., 1996). In spite of all these influences, it has also been demonstrated that elimination of renal innervation does not have to lead to changes of glomerular filtration rate (Stella and Zanchetti, 1977; Pelayo et al., 1984), possibly as a result of relaxation of both afferent and efferent arteriole.

Nitric oxide synthetase isoforms expressed in the renal medulla have a potent influence on renal medullary, tubular and vascular function, with consequential effects on fluid and electrolyte homeostasis and arterial blood pressure (Mattson and Wu, 2000). The increase in medullary nitric oxide production associated with the activation of α_2-adrenergic receptors, counteracts the vasoconstrictor effects of norepinephrine in the medulla and may play an important role in maintaining a constancy of medullary blood flow and oxygenation (Zou and Cowley, 2000).

The arrangement of the renal microcirculation suggests various sites at which modulation of vascular tone could control regional perfusion. Constriction of intralobular arteries might

distribute blood flow towards the medulla by favoring perfusion of juxtamedullary glomeruli. Outer medullary descending vasa recta vasodilatation by adenosine involves cAMP and cyclooxygenase (COX), but not nitric oxide (Silldorff and Pallone, 2001). On the other hand, closure of juxtamedullary afferent or efferent arterioles might favor perfusion of the superficial cortex. Increased breakdown of bradykinin by angiotensin converting enzyme favors cortical blood flow rather than medullary blood flow (Omoro et al., 1999; Omoro et al., 2000).

The ultimate goal of renal function is the ultrafiltration of plasma at the level of the glomerulus and subsequently processing this ultrafiltrate by the tubular system to eventually meet the targets of excretion of waste products as well as keeping the balance between water (salt) input and output. As has been stated earlier, the rate of this ultrafiltration or glomerular filtration rate (GFR) depends largely on local hemodynamic factors i.e. vasomotion of the afferent and/or efferent arteriole.
Secondly, GFR can also be affected by a change in glomerular capillary filtering surface area and hydraulic permeability of the filtration barrier. This can be accomplished by contraction of the mesangial cells and both angiotensin II and arginine vasopressin seem to be regulators at this level (Schor et al., 1981). It has also been demonstrated that locally produced nitric oxide controls the hydraulic permeability barrier (Deng and Baylis, 1993).
Moreover, ultrafiltration will be the net result of the Starling forces locally present, i.e. hydrostatic and colloid osmotic pressure in the glomerular capillaries and the same pressures in the capsule of Bowman. These are physical factors that depend on various parameters such as renal blood flow, tubular outflow and tubular absorption rate (Dworkin and Brenner, 2000; Maddox and Brenner, 2000).

1.1.2 Sodium and water

1.1.2.1 General

In the conscious rabbit about 5 - 8 l of nearly protein-free ultrafiltrate is formed daily and of this amount only a small fraction is excreted in the form of urine (Kozma et al., 1974; Sejersted, 1977). The renal tubules process this large volume of filtrate to conserve the essential nutrients (glucose, amino acids, and metabolites of different biochemical pathways), to eliminate potentially toxic substances (organic acids and bases, K^+, acid, nitrogenous waste products), and to reduce the quantity of salt and water excreted in the

final urine. The conservation of the essential nutrients, such as glucose and amino acids, occurs primarily in the first part of the proximal tubule, whereas the secretion of organic ions and cations occurs in more distant parts. The fluid leaving the proximal tubule under normal conditions can reach 40% to 50% of the volume filtered and is predominantly an iso-osmotic NaCl solution containing small amounts of K^+, Ca^{2+}, Mg^{2+}, $H_2PO_4^-$, HPO_4^{2-}, HCO_3^-, urea and secreted organic substances. In the more distal part of the nephron, the reabsorption of NaCl is the predominant transport process and is coupled with other tubule transport functions: excretion of water as a dilute urine, conservation of water through production of a concentrated urine, reabsorption of Ca^{2+}, and Mg^{2+}, and secretion of K^+ and acid (Rector, 1983; Moe et al., 2000).

Tubules are lined by epithelia and these tissues have the unique capacity to transport solutes and water from one surface to another. This vectorial transport (one direction) is a direct consequence of the polarization of the epithelial cell into distinct apical and basolateral membranes (Spring, 1998).

The basolateral membrane possesses the ubiquitous primary active transport mechanism, the Na^+, K^+ - ATPase pump. The Na^+, K^+ - ATPase pumps Na^+ out of the cell into the peritubular compartment in exchange for peritubular K^+. The activity of the pump maintains the cellular concentrations of Na^+ lower and of K^+ higher than their respective concentrations in ambient fluids and couples sodium transport in the nephron directly to metabolic energy. Although there are other ATPases in the cell, the Na^+, K^+ - ATPase energizes the majority of solute transport in the mammalian kidney. All basolateral Na^+ transport along the nephron is dependent on the Na^+, K^+ - ATPase, which is distributed on the basolateral side of the entire (rabbit) nephron (El Mernissi and Doucet, 1984; Feraille and Doucet, 2001).

The apical membrane possesses either diffusional or carrier-mediated mechanisms for the transport of Na^+ and various ions and solutes. The localization of the Na^+, K^+ - ATPase exclusively at the basolateral membrane, and the localization of the carrier mediated, Na^+ coupled transport mechanisms at the apical membrane, enables the individual nephron segments to transport ions, solutes and water vectorially from lumen to blood. The net flux of Na^+ from lumen to peritubular fluid varies among the different nephron segments and correlates with the amount of Na^+, K^+ - ATPase in the basolateral membrane (Feraille and Doucet, 2001).

As a consequence of the anatomy of epithelia, two distinct transepithelial transport pathways are arranged in parallel. The transcellular pathway, which is serially made up of the luminal or apical membrane, the cell cytoplasm and the basolateral or peritubular cell membrane and the paracellular pathway made up of the junctional complexes and the intercellular spaces arranged in series. Transport through the cell generally requires energy; transport between the cells does not. The basement membrane does not present a significant barrier to solute or solvent movement (Kiil, 2002b).

Transport of solutes such as sugars and amino acids across biological membranes requires a variety of specialized transporters. These membrane molecules are responsible for creating and maintaining the appropriate compositions of the cell interior and for sustaining life in response to changes in the extracellular environment.

Table 1.1: Major sodium transporter and channel proteins in the kidney.

Name	Identification	Location
NHE3	Type 3 Na-H exchanger	PT, DL, TAL (apical)
NaPi-2	Type 2 Na-Phosphate cotransporter	PT (apical)
NBC 1	Type 1 Na-bicarbonate cotransporter	PT (basolateral)
NKCC2	Type 2 Na-K-2Cl cotransporter	TAL (apical)
NCC	Na-Cl cotransporter	DCT (apical)
ENAC	Epithelial sodium channel	CNT, CD (apical)
NKA	Na-K-ATPase	All segments (basolateral)

PT, proximal tubule; DL, descending limb of Henle's loop ; TAL, thick ascending limb of Henle's loop; DCT, distal convoluted tubule; CNT, connecting tubule; CD, collecting duct. Modified from: (Knepper and Brooks, 2001)

Transporters can be divided into passive and active transporters. Passive transporters allow diffusion across membranes down their electrochemical gradient. Similar to transporters, channels allow movement of solutes down their electrochemical gradients (Hall and Guyton, 2000; Moe et al., 2000). A lot of the knowledge about these transporters was derived from the use of transgenic or specific gene knocked-out mice (Rao and Verkman, 2000). Table 1.1 presents an overview of different sodium transporters and their (specific) location in the nephron.

NaCl reabsorption occurs almost along the entire nephron and this follows a general rule: Na^+ entry across the apical membrane is the primary determinant of intracellular Na^+ concentration in the epithelial cells. In turn, intracellular Na^+ concentration directly controls the activity of the Na^+,K^+-ATPase that is extruding Na^+ at the basolateral side. Therefore, apical Na^+ entry is the limiting factor for transepithelial Na^+ and fluid transport and any change in the quantity and/or the activity of these transporters should affect the reabsorption rate (Meneton et al., 2001). For instance, a defect in the Na^+-Cl^- cotransporters (NCC) in the distal convoluted tubule would result in a mild salt wasting disorder called Gitelman syndrome (table 1.2) (Schnermann, 2000).

1.1.2.2 The proximal tubule

The proximal tubule accounts for about 65% of total renal and water reabsorption. Sodium reabsorption in the early part of the proximal tubule is principally mediated through the sodium-solutes cotransporters (phosphate, glucose and amino acids) and the sodium-proton exchanger type 3 (NHE3). In the early proximal tubule Na^+ and HCO_3^- reabsorption are coupled due to the activity of the apical Na^+/H^+ exchanger (1), the basolateral Na^+/K^+ ATPase (2), potassium channels (3) and the Na^+/HCO_3^- cotransporter (4). Extrusion of protons out of the cells (1) leads to alkalinisation of the cytoplasm. This in turn favours the extrusion of bicarbonate, at the basolateral side, with a concomitant transport of sodium ions (4). Generation of H^+ and HCO_3^- is catalysed by the enzyme carbonic anhydrase, which is present in the intracellular space as well as on the apical surface of the epithelial cells. Specific pharmacological agents can antagonise this enzyme and as a result of the decreased availability of H^+ result in a decreased Na^+ reabsorption. In this way these agents serve as diuretic agents (Preisig et al., 1987). However, the effect of these drugs is not pronounced and (genetic) defects of the NHE3 exchanger (1) only lead to minor salt wasting syndromes (Schnermann, 2001). Decreased proximal reabsorption of Na^+ leads to an increased delivery of Na^+ to the distal part and macula densa of the nephron. In this way the tubuloglomerular feedback is activated and GFR will decrease, resulting in less sodium load. Moreover there appears a compensational increase of Na^+ transporters more distally in the nephron. Thus salt excretion is less than expected in the above cases (Lorenz et al., 1999; Bernardo et al., 1999; Schnermann, 2000; Meneton et al., 2001; Knepper and Brooks, 2001).

The reabsorption of sodium mediated through the solute cotransporters renders a lumen negative potential difference. This potential difference favours possible paracellular chloride reabsorption. Quantitatively marked differences exist along the tubule. Reabsorption of sodium, water, glucose and HCO_3^- in the early proximal tubule is about three-fold greater than that of the midportion of the convoluted proximal tubule and nearly ten times that of the straight segment of the tubule (Moe et al., 2000; Weinstein, 2000; Knepper and Brooks, 2001).

As a result of the preferential secondary active absorption of filtered sugars, amino acids, $NaHCO_3$, and sodium salts of other organic anions in the initial parts of the proximal tubule, the fluid that enters the remaining parts of the proximal tubule has a Cl^- concentration higher than that in peritubular plasma. This high Cl^- composition denotes the second phase of proximal reabsorption, which consists of reabsorption of NaCl predominantly and only slight net reabsorption of neutral organic solutes and nonchloride sodium salts. Both active and passive Na^+ and Cl^- transport processes contribute to NaCl reabsorption in the second phase of proximal reabsorption (Vari and Ott, 1982; Wong et al., 1986; Weinstein, 2000).
As tubular fluid flows along the proximal tubule, its pH and bicarbonate concentration decrease as a consequence of the reabsorption mechanisms in the early part. Luminal acidification reduces the efficiency of the NHE3 because of a possible inverse proton gradient. Therefore Na^+ uptake via the luminal membrane is indirectly coupled to Cl^- and becomes independent of HCO_3^- exit via the basolateral membrane. The absorption of NaCl in the proximal tubule besides $NaHCO_3$, is markedly stimulated by formate and oxalate. This is consistent with the presence of two processes involving Cl-formate and Cl-oxalate exchange in parallel with recycling of formic and oxalic acid across the apical membrane. In the case of formate stimulated NaCl reabsorption it has been proposed that a pH-coupled formate recycling is present due to non-ionic diffusion of formic acid and/or H^+ - formate cotransport. These processes in turn require H^+ secretion in parallel with Cl-formate exchange. The H^+ secretion is mediated by NHE3. In NHE3 null mice formate added to the luminal perfusion solution fails to increase J_{Cl} significantly. In contrast, the stimulation of J_{Cl} by 5 μM oxalate is similar between wild–type and NHE3 null mice. This finding indicates that oxalate stimulated NaCl reabsorption is independent of Na-H exchange (Wang et al., 2001; Knauf et al., 2001). The transport is mediated by Na-sulfate cotransport, sulfate-oxalate exchange in parallel with Cl-oxalate exchange. In the presence of basolateral exit

pathways for chloride (chloride channels and potassium chloride cotransporters), reabsorption of chloride occurs transcellularly and therefore chloride and sodium transport are indirectly coupled (Liapis et al., 1998). In rat proximal tubules it is demonstrated that a small fraction of the Na^+ reabsorption could be mediated through ENaC channels (Willmann et al., 1997).

In the early proximal tubule, solute reabsorption proceeds faster than solvent reabsorption, generating relative lumen hypotonicity and intercellular hypertonicity. This relative osmotic gradient is responsible for passive fluid reabsorption (Moe et al., 2000; Kiil, 2002a).
Aquaporin-1 is the dominant pathway for transmembrane water fluxes across both the apical and basolateral membranes. It is a member of a family of 10 proteins cloned from mammals. The aquaporins are small very hydrophobic intrinsic membrane proteins that permit the passage of water. In the kidney at least seven aquaporins (AQP) are expressed at distinct sites. AQP-1 is present in the proximal tubule and in the thin descending limb of Henle and is essential for urinary concentration. AQP-2 is exclusively present in the principal cells of the connecting tubule and collecting duct and is the predominant vasopressin-regulated water channel. AQP-3 and AQP-4 are both present in the basolateral plasma membrane of the principal cells in the collecting duct and form the exit pathways for water that entered the cells at the apical membrane (Bondy et al., 1993; Van Os et al., 2000; Agre et al., 2000; Kwon et al., 2001).

AQP-1 is responsible for about 80% of the water conductivity of the proximal tubule. In AQP-1 knocked out mice, luminal osmolality decreased in the distal parts of the proximal tubule. However water reabsorption was reduced only by 50%. These results indicated that AQP-1 plays a major role in osmotic reabsorption of water, but other pathways like the paracellular route might also contribute to this transport (Vallon et al., 2000; Schnermann, 2001). On the other hand, Jacobson and coworkers had already indicated that there was a lack of solvent drag in rabbit nephrons and that the major route of water transport might be transcellular (Jacobson et al., 1982).
As is true for the NHE3 proteins, possible defects in AQP-1 are probably compensated by reductions in glomerular filtration and adjustments of transport in the terminal nephron (Schnermann, 2001). A summary of possible defects in transporters and their related syndromes is presented in table 1.2.

Table 1.2: Summary of transporter defects and their severity in Na wasting syndromes.

Transporter defect	Location	Human disease syndrome	Na wasting in the newborn	Na wasting Adult	GFR reduction
AQP1	PT	Colton -/-	Mild	Mild	+
NHE3	PT	None known	Mild	Mild	+
NKCC2	TALH	Antenatal Bartter	Severe	Less Severe	-
ROMK	TALH	Antenatal Bartter	Severe	Less Severe	-
ClC-Kb	TALH	Classic Bartter	Less Severe	Less Severe	-
NCC	DCT	Gitelman	Very Mild	Very Mild	+/-
ENaC	CD	Pseudohypoaldosteronism 1	Severe	Less Severe	-

AQP1, aquaporin 1; NHE3, Na/H exchanger 3; NKCC2, Na-K-2Cl cotransporter 2; ROMK, K channel; ClC-Kb, thick ascending limb Cl channel; NCC, Na/Cl cotransporter; ENaC, epithelial Na channel; PT, proximal tubule; TALH, thick ascending limb of Henle's loop; DCT, distal convoluted tubule; CD, collecting duct. Modified from (Schnermann, 2000)

In vivo many hormones and neurotransmitters, including PTH, dopamine, epinephrine and norepinephrine and angiotensin II, control sodium and fluid reabsorption in this part of the nephron. PTH is the main regulator of phosphate transport, but bicarbonate reabsorption is also affected and most likely it plays a role in the overall sodium and fluid balance. The major part of sodium and water reabsorption is increased by angiotensin II and (nor)epinephrine and decreased by dopamine. Depression of renal nerve activity reduces proximal reabsorption of both sodium bicarbonate and sodium chloride. It has also been demonstrated that this reduction is independent of changes in filtration rate, since they do not occur (Pelayo et al., 1984; Cogan, 1986; Baum and Quigley, 1998; DiBona and Kopp, 2000; Feraille and Doucet, 2001).

Thus water and solute transport in the proximal tubule can be accomplished in the following ways: a transcellular one and a paracellular one, in which solutes can be transported by means of diffusion or as a consequence of solvent drag. Recent findings such as hypotonicity of the proximal tubule fluid in aquaporin-1 knocked out mice and lack of solvent drag in rabbit proximal tubules however, favour the first route more (Jacobson et al., 1982; Vallon et al., 2000).

1.1.2.3 The loops of Henle

The thin descending and ascending limbs of Henle have a major contribution to the process of urinary concentration and dilution, mostly by passive transport of NaCl and water. The

descending limbs of Henle have extremely low Na^+, K^+ - ATPase activity and are probably not capable of significant active transport. Water permeability is generally high (AQP-1) and solute permeability is low. Some NaCl is dragged by water flow from lumen to interstitium and some NaCl diffuses down its concentration gradient from medullary interstitium to tubule fluid. Tubule fluid in the descending limb of Henle concentrates mostly by abstraction of water. This process will extensively be discussed in subsequent paragraphs on renal concentrating mechanisms (Chou et al., 1999b; Moe et al., 2000). The thin ascending limb of Henle is similar to the thin descending limb in that it has a flat endothelium-like epithelium without signs for significant Na^+, K^+ - ATPase activity, thus no active transport of solutes. In contrast to the descending limb, there is no permeability for water, and a high permeability to NaCl. A specialized chloride channel (CLC-K1) at the basolateral plasma membrane of these cells allows passive Cl^- permeability transcellularly and plays a major role in the NaCl reabsorption at that site. Sodium diffuses via a paracellular pathway. Transport processes in this part of the loop of Henle are described extensively in the subsequent paragraphs on renal concentrating mechanisms and will not further be discussed here (Reeves and Andreoli, 2000; Takahashi et al., 2000; Reeves et al., 2001; Akizuki et al., 2001).

Cl^- transport in the loop of Henle is responsible for reclamation of about 25% of the filtered NaCl load and for the formation of dilute urine. About 15% is secondarily active reabsorbed by the thick ascending limb of Henle. Because the thick ascending limb is impermeable to water, the reabsorption of NaCl always dilutes the tubular fluid, independent of the state of the water balance. This reabsorption of NaCl from the tubule fluid into the interstitium followed by a very mild water flow plays an important role in the generation of both concentrated and dilute urine (Reeves and Andreoli, 2000; Reeves et al., 2001).
The sodium gradient generated by the basolateral Na^+,K^+-ATPase in this part of the nephron is mainly dissipated by an apical electroneutral Na^+-K^+-$2Cl^-$ cotransport system that couples downhill entry of sodium to the uphill transport of potassium and chloride. The loop diuretics like furosemide and bumetanide specifically inhibit this cotransport system (Greger and Wangemann, 1987; Unwin et al., 2000).
Potassium ions accumulated in the cell above Nernst equilibrium by this transporter, exit the cells via the apical ROMK, special inwardly rectifying, voltage-insensitive potassium

channels. This transport of potassium to the lumen is required to feed the cotransporter with potassium ions. Failure of this process blocks the cotransporter.
Chloride leaves the cells across the basolateral membrane via special (ClCK2) channels and electroneutral K^+-Cl^- cotransporters (KCCl) (Liapis et al., 1998; Reeves et al., 2001).
This conductive diffusion of chloride and potassium depolarizes the basolateral membrane and hyperpolarizes the apical membrane, respectively. These two diffusion potentials in series combine to create a lumen-positive transepithelial voltage characterizing the thick ascending limb. This positive potential difference serves as a driving force for paracellular cation (Na^+, K^+, Ca^{2+} and Mg^{2+}) reabsorption. The maintenance of a constant intracellular sodium concentration requires that all three transporters mentioned previously function in an adequate way. Dysfunction of either of these three may lead to Bartter's syndrome, a salt wasting disorder (Schnermann, 2000; Russell, 2000; Takahashi et al., 2000; Feraille and Doucet, 2001; Schnermann, 2001; Meneton et al., 2001).

Sympathetic stimulation via β-adrenergic receptors will increase NaCl reabsorption in the TAL via an increase in cellular cAMP. Vasopressin also stimulates the reabsorption of NaCl in this part of the nephron by increasing the cAMP content of the cells. In fact cAMP plays a central role in the regulation by hormonal and neuronal stimuli. It means that decreasing the stimulus of one factor does not necessarily lead to decrease of cellular cAMP content, due to the remaining action of the other stimuli and, therefore, the effects will remain minimal (DiBona and Kopp, 2000; Feraille and Doucet, 2001).

1.1.2.4 Distal tubule and collecting duct

The distal convoluted tubule of the rabbit creates a lumen negative potential difference that is ouabain sensitive (Moe et al., 2000). It reabsorbs about 5% of the filtered sodium load and is relatively water impermeable. NaCl uptake by the cells occurs mainly through the apical NaCl cotransporter (NCC). The expression of NCC is regulated by mineralocorticoids. Thiazide diuretics can block these NCC transporters and induce sodium wasting in this part of the nephron. Transport defects of these proteins may lead to Gitelman's syndrome (Kim et al., 1998; Schnermann, 2001). The connecting tubule and the initial collecting tubule segments are the important sites of fine-tuning of the composition of the urine. A very small part (2%) of the filtered NaCl is reabsorbed here under the influence of aldosterone.

In spite of this small part of the total NaCl reabsorption, the collecting segments are crucial in deciding how much NaCl is eventually excreted (Moe et al., 2000). NaCl reabsorption across the inner medullary collecting duct is normally under influence of arginine vasopressin. Absorption of sodium in this part of the nephron is apically mediated by amiloride sensitive Na^+ channels (ENaC). Both aldosterone and arginine vasopressin influence the reabsorption in this part via regulation of ENaC (Stanton, 1989; Kudo et al., 1990; Ling et al., 1991; Schnermann, 2001; Ecelbarger et al., 2001).

1.1.2.5 The concept of Glomerulotubular Balance

It has long been recognised that tubular factors were not the only ones involved in NaCl and water reabsorption, but that peritubular factors also played a major role in the recovery of fluid and salt. Elevation of the venous pressure in the renal vein decreased proximal tubular reabsorption. This effect could be neutralised by elevation of the oncotic pressure in the peritubular capillaries, implying that capillary flow rate and colloid osmotic pressure could influence proximal tubular reabsorption (Windhager et al., 1969).

The renal regulation of the volume and the composition of body fluid compartments depends largely upon the capacity of the tubular epithelium to adapt its rate of sodium transport to changes in glomerular load. Immediate and autonomous adjustments of tubular reabsorption to variations in GFR are a prerequisite for maintenance of constant volume and composition of body fluids (Haberle et al., 1981b; Kiil, 1982). It is well established that almost 90% of the ultrafiltrate is being reabsorbed by the proximal tubule and the thick ascending limb of Henle, the remaining 10% being handled by the distal tubule and collecting duct segments of superficial nephrons of the rabbit (Wong et al., 1986). The existing proportionality between proximal tubular reabsorption and GFR is called glomerulotubular balance (GTB). GTB is a fundamental property, since the variation in GFR of $\pm$ 50% results in similar changes in proximal tubular reabsorption (Kiil, 1982). Flow rate, tubular cross section and variations in hydrostatic and colloid osmotic pressure across the proximal tubular wall and peritubular capillaries are proposed as mechanisms mediating GTB (Bank, 1979). Peritubular oncotic forces are generally recognized as a major force in the maintenance of balanced tubular reabsorption. Any increase in filtration fraction must result in an increased peritubular protein concentration and thus an increased reabsorption (Weinstein, 2000).

On the other hand intraluminal and metabolic factors are also being considered as factors in GTB. Facilitated transport across tubular membranes would, as in other enzymatic processes, be expected to follow Michaelis Menten kinetics. By raising the substrate concentration, the rate of membrane passage would increase in proportion until all carrier proteins become saturated. For a solute with declining concentration along the nephron, a rise in GFR would increase the concentration and thus stimulate its transcellular reabsorption (Kiil, 1982). This concept of GTB seems more probable, since it is clear that the intracellular sodium controls the rate of the Na-K-ATPase and hence the rate-limiting factor becomes the apical transport of sodium and fluid (Stanton and Kaissling, 1989; Feraille and Doucet, 2001). An increased concentration of luminal substrates will increase the function of sodium coupled transporters through Michaelis Menten kinetics. That means that the transport rate will increase linearly with substrate concentration within a certain interval of concentration and saturate above a certain level. Both tubuloglomerular feedback and glomerulotubular balance control the ultimate salt delivery to the distal tubule and salt excretion in urine. This arrangement prevents small changes in GFR or inefficiencies in reabsorption from resulting in large changes in solute and water excretion (Thomson et al., 1999).

1.1.2.6 Pressure diuresis and natriuresis

Pressure natriuresis refers to the effect of increased blood pressure to raise sodium excretion. Under most conditions this mechanism acts to stabilize arterial pressure and body fluid volumes. An increase in peripheral vascular resistance or in cardiac output (fluid intake) for instance will tend to increase blood pressure. With normal kidney function, there will be pressure natriuresis and diuresis resulting in a decrease of the intravascular volume. As long as renal excretion exceeds fluid intake, extracellular fluid volume will continue to decrease, reducing venous return and cardiac output until blood pressure returns to normal and fluid intake and output are balanced again. On the other hand, when blood pressure decreases, the kidneys retain salt and water until arterial pressure is restored to normal. In this way pressure natriuresis acts as a key component of the feedback system that normally serves to stabilize blood pressure and body fluid volumes.

In the intact animal pressure diuresis can be influenced by numerous factors other than perfusion pressure alone. The effect of blood pressure on diuresis can be blunted or amplified by various neuro-humoral factors. For example, a minimal increase of blood

pressure may result in a decrease of aldosterone level and thereby increasing salt and water excretion. On the other hand, increased catecholamines will decrease salt excretion and require a higher blood pressure to excrete the same amount of NaCl and fluid (Guyton, 1990, 1991; Hall et al., 1996; Guyton and Coleman, 1999). Therefore it is ideal to investigate the phenomenon of pressure diuresis with isolated kidney preparations. Extrarenal compensatory factors can selectively be eliminated or amplified in this system.
To accentuate the importance of this, Gleim and co-workers reported pressure natriuresis on an isolated rat kidney as a result of an increase in filtered sodium load and a decrease of fractional sodium reabsorption. Inhibition of prostaglandin synthesis led to a rightward shift of the renal function curve (Gleim et al., 1984). In a conscious animal, one would not be able to inhibit prostaglandin synthesis without affecting systemic and renal hemodynamics with resulting alterations in kidney function. For example, the release of atrial natriuretic factor cannot be omitted.

The exact molecular mechanism that underlies pressure diuresis and natriuresis is still obscure. Redistribution of the sodium hydrogen isoform 3 exchanger out of the apical membrane and/or downregulation of the Na-K-ATPase activity might play a role during acute hypertension (McDonough and Biemesderfer, 2003; McDonough et al., 2003) or increase of renal interstitial hydrostatic pressure (Granger et al., 2002), that might be a consequence of increased renal medullary blood flow (Roman and Zou, 1993).

1.1.3 Phosphate

Renal handling of phosphate determines its concentration in the extracellular space. To fulfil the homeostatic function of keeping extracellular phosphate concentration within a narrow range, urinary phosphate excretion must be under strong physiological control and renal phosphate excretion can indeed adjust very fast to alterations in the phosphate concentration.
Renal phosphate excretion is the balance between free glomerular filtration of phosphate and regulated tubular reabsorption. Approximately 80% - 90% of the phosphate load is reabsorbed in the proximal nephron. Probably there is little or no reabsorption of phosphate in the remaining part of the nephron, making its fractional reabsorption a fine marker of proximal tubule function.

Phosphate is taken up from the tubular fluid by brush-border membrane sodium/phosphate cotransporters and leaves the cell via basolateral transport pathways. The brush-border entry step is the rate-limiting step. Basolateral exit is not yet established and sodium/phosphate cotransporters, anion exchangers or even a phosphate leak channel have been considered as possible transport mechanisms. The proximal tubule cell has to complete transcellular phosphate reabsorption in cases where luminal phosphate entry exceeds the metabolic requirements of this anion (Moe et al., 2000).

Phosphate handling by the kidney is under strong hormonal control. Parathyroid hormone (PTH) induces phosphaturia by inhibiting brush-border membrane Na-Pi cotransport activity. Vitamin D is suggested to increase/stimulate proximal tubular Pi reabsorption. 1,25-Dihydroxycholecalciferol treatment of rats was found to stimulate brush-border membrane Na-Pi cotransport. Insulin enhances proximal tubular Pi reabsorption by stimulation of brush-border membrane Na-Pi cotransport and prevents the phosphaturic action of PTH. Thyroid hormone stimulates proximal tubular Pi reabsorption via a specific increase in brush-border membrane Na-Pi co-transport, while calcitonin reduces this transport. Apart from these hormonal influences plasma calcium and pH of proximal tubular cells may also play a role (Quamme et al., 1985; Murer et al., 2000; Silve and Friedlander, 2000; Suki et al., 2000).

1.1.4 Glucose

Glucose is freely filtered through the glomerular barrier and is completely reabsorbed in the proximal tubules. Were it not for this reabsorption, the organism would be losing a lot of organic fuel in the urine. Clearance studies of glucose may then be used as markers of proximal tubule functionality.

Glucose is transported over the luminal and basolateral membrane with the assistance of sodium dependent (SGLT), respectively non-sodium dependent (glut) transporters. It is thus reabsorbed from the luminal fluid via a secondary active transport mechanism and leaves the cells at the basolateral border through facilitated diffusion.
In the first part of the proximal tubule, there are low affinity, high capacity transporters built in both membranes (SGLT-2 and glut2). The bulk of the glucose load is then reabsorbed. Further in the tubule there are high affinity, low capacity transporters that are

able to pick up the already low concentrated glucose in the tubule and transport it through the apical as well as the basolateral membrane (SGLT-1, respectively glut1). This configuration makes sure that in the first place all filtered glucose is reabsorbed. Secondly it prevents that the initial proximal tubule is loaded with large amounts of glucose that must be reabsorbed (Berger et al., 2000; Moe et al., 2000; Silverman, 2000; Wright, 2001).
Because the glucose is reabsorbed exclusively via these transporters, it is clear that this enzymatic reabsorption will express a maximal transport capacity.
It is also clear that an increase in glucose reabsorption via the SGLT transporters will increase the sodium transport as well. This means that less NaCl will reach the Macula Densa and that GFR will consequently rise with an increase in glucose load. Indeed it has been observed that in the initial phase of Diabetes Mellitus there is a glomerular hyperfiltration (Vallon et al., 1999; Moe et al., 2000).

1.1.5 Overview of potassium transport in different nephron segments

The kidneys play a major role in potassium balance in the body by increasing the output of excess K^+ in case of a high input and reducing output to almost zero with decreased intake (Giebisch and Wang, 2000; Reilly and Ellison, 2000; Muto, 2001).
There are various channels and transporters along the whole nephron that are involved in the renal response which is slow and requires several hours (Giebisch, 1998). Not only do K^+ channels play an important role in the process of K^+ secretion, they are also involved in the mechanism by which the tubule cells maintain fluid and electrolyte equilibrium.

Firstly, K^+ channels participate in the generation of the cell-negative potential that provides an important driving force for the movement of charged solutes across both apical and basolateral membranes of tubule cells. However this process is encountered in all mammalian cells and is not unique to the kidney.
Secondly, K^+ channels are involved in the regulation of the volume of tubule cells. They control the movement of K^+ into and out of the cells, minimizing changes in cell volume when cells are exposed to an anisosmotic environment and when an increase in solute and fluid transport involves rapid entry of solutes. Like the previous mechanism, this process is also not unique to kidney cells and can be found in almost every other mammalian cell (Van Driessche et al., 1997).

Thirdly, the regulation of the body's K^+ balance depends on the secretion of K^+ by the principal cells in the collecting duct, and the secretion occurs through apical K channels. Of course this process is exclusively maintained by the kidney (Giebisch and Wang, 2000).

1.1.5.1 K^+ transport in the proximal tubule

Following filtration, potassium is extensively reabsorbed along the proximal tubule and the loop of Henle. In rats, only 10% of the filtered load of potassium reaches the initial distal tubule (Giebisch and Wang, 2000; Malnic et al., 2000). However, Wong and co-workers found that this value was significantly different in superficial nephrons of rabbits and valued about 50% (Wong et al., 1986). This difference could be the result of potassium recycling in the medullary interstitium, since K^+ is partly reabsorbed in the inner stripe of the outer medullary collecting duct and in the thick ascending limb of Henle's loop and consequently results in a high medullary interstitial [K^+] that enters the pars recta and descending thin limb of Henle's loop (Muto, 2001).

Several K^+ channels have been identified in the basolateral membrane. These K channels are not only responsible for the generation of the cell-negative potential, but their sensitivity to the inhibitory action of different intracellular substances has implicated them in several functions. Inhibition of basolateral K^+ channels by ATP is thought to play a central role in the tight coupling between Na^+-K^+-ATPase activity and K^+ conductance (Tsuchiya et al., 1992; Wiehart et al., 2003).

Besides the ATP sensitive channels on the basolateral membrane, there are also stretch sensitive and voltage sensitive K^+ channels on this side. Keeping the large cellular negativity will maintain the electrochemical driving force for secondary active transport of solutes, which is probably the most important function of these channels (Wang et al., 1997; Giebisch, 1998; Giebisch, 2001).

Apical K^+ channels are characterized by low open probability under normal physiological conditions but may become active during cell volume expansion, membrane depolarisation, and an increase in cytosolic Ca^{2+} concentration. These channels are thought to be involved in stabilization of the apical potential under conditions that tend to depolarize proximal cells, for instance, during enhanced electrogenic cotransport involving sodium-dependent organic solute transport. Electrophysiological studies in perfused proximal tubules indicated that the K^+ flux through these apical channels is essential to counteract membrane

depolarization due to electrogenic Na^+ coupled transport of amino acids and glucose. In mice, knocked out of these channels, fractional excretion of Na^+, Cl^-, fluid and glucose was significantly higher (Vallon et al., 2001).

1.1.5.2 K^+ transport in the thick ascending limb (TAL) of Henle's loop

Remarkable in the TAL is the presence of an apical recycling of potassium in this part of the nephron. This recycling is necessary, for the amount of K^+ entering the TAL is too small to keep NaCl reabsorption via the NaK2Cl transporters in the apical membrane at the appropriate level. Three different channels have been identified with different regulatory mechanisms.

Recycling of potassium at the apical membrane of these cells indicates that the transport of this ion in this part of the nephron, plays more a regulatory role in the reabsorption of sodium. Potassium reabsorption may be replaced by potassium secretion when the apical Na-K-2Cl cotransporter is inhibited, for instance by loop diuretics. Removal of potassium from the lumen or inhibition of the apical potassium conductance leads to a sharp decline of NaCl reabsorption (Bartter's syndrome). The apical potassium conductance is also essential for the generation of the lumen positive potential difference, which provides a significant driving force for passive cation reabsorption (i.e. Ca^{2+} and Mg^{2+}) along the paracellular transport pathway, as has already been explained earlier in this chapter.

A low-potassium diet, aldosterone and vasopressin stimulate potassium reabsorption in the thick ascending limb of Henle's loop, while a high potassium intake and high plasma calcium reduce reabsorption (Giebisch, 1998; Giebisch and Wang, 2000).

1.1.5.3 K^+ transport in collecting duct

The collecting duct is involved in the regulation of potassium excretion by the kidney. The principal cells mainly accomplish potassium secretion, while the intercalated cells are more involved with reabsorption. After raising basolateral [K^+] by higher intake of K^+ or a shift of intracellular K^+ to the extracellular space, the electrogenic Na^+, K^+-ATPase is stimulated. This stimulation leads to a series of secondary effects: intracellular [Na^+] falls, intracellular [K^+] increases, the ATP/ADP ratio decreases, due to consumption of ATP by the pump, and the membrane potential difference increases in absolute values after initial depolarisation. These secondary effects elicit another series of events: increase in cellular pH (via stimulation of Na-H exchanger), decrease of intracellular [Ca^{2+}] (via stimulation of Na-Ca

exchanger) and as a consequence of the decrease in [Ca^{2+}], a decrease in protein kinase C (PKC) activity. All these events lead to opening of the apical Na^+ channel (ENaC), and the decrease in ATP/ADP, [Ca^{2+}] and PKC open the apical K^+ channel. Intracellular increase of K^+ and decrease of Na^+ lead to an enhanced efflux of K^+ and influx of Na^+ on the luminal side of the cells and results in an increased secretion of potassium. Opening of the potassium channel is also depending on the apical potential difference, which varies with the Na^+ concentration difference between luminal and intracellular [Na^+]. On the other hand, a decrease in plasma [K^+] will eventually lead to closure of both channels, resulting in decreased secretion of K^+ and decreased reabsorption of Na^+ (Giebisch, 1998; Muto et al., 1999; Palmer, 1999; Giebisch and Wang, 2000; Giebisch, 2001).

The kidney's ability for potassium reabsorption in distal nephron segments has been localized at the intercalated cells in the initial and cortical collecting tubule and at cells in the inner stripe of the outer medullary collecting duct. Reabsorption is mediated by apical H^+-K^+-ATPases. Potassium depletion induces recruitment and insertion of potassium absorbing ATPases in the apical membrane (Giebisch, 1998).

1.1.5.4 Control of potassium transport in the collecting duct

Potassium excretion by the kidneys is influenced by the following factors:

– Rate of distal fluid and Na^+ delivery: enhanced delivery of fluid and Na^+ to the distal tubule is one of the most powerful and frequently activated mechanisms of distal tubule K^+ secretion. Sustained diuresis often leads to severe K^+ depletion. Two separate factors contribute to the markedly enhanced rate of K^+ secretion following enhanced delivery of fluid and Na^+ into the distal tubule. First, when flow increases in the distal tubule, the luminal K^+ concentration decreases moderately. However, the reduction of luminal potassium concentration is proportionally smaller than the increase in flow rate, thereby increasing K^+ secretion. The decline in luminal K^+ concentration will steepen the chemical concentration gradient of K^+ across the apical membrane and facilitate K^+ secretion into the lumen. Basolateral K^+ uptake must increase sharply to maintain cell K^+ concentration. The second factor responsible for increased K^+ secretion, associated with enhanced flow rates, may be the elevation of luminal Na^+ concentrations. During natriuresis, Na^+ delivery into the distal nephron enhances and increases Na^+ entry into cells across the apical membrane. It facilitates the basolateral Na^+-K^+ exchange, and

thereby increased Na^+ reabsorption occurs. As a result, cell K^+ concentration increases, stimulating the movement of K^+ from cell to lumen. Also, increased Na^+ entry into the cell across the apical membrane causes the apical membrane to depolarise, facilitating an enhanced K^+ secretion across this membrane.

- Adrenal steroids: chronic exposure to mineralocorticoids in vivo results in an increase of sodium reabsorption, potassium secretion and lumen-negative potential. The first effect after administration of mineralocorticoids is an increase in apical membrane Na^+ conductance. A secondary delayed effect is the increase in K^+ conductance of the apical membrane while also an increase in Na-K-ATPase activity can be measured. Glucocorticoids can increase K^+ excretion as well.
- K^+ intake: after an increase in K^+ intake, direct and indirect effects of hyperkalemia stimulate potassium secretion by the distal tubule and cortical collecting duct. As has already been emphasized, an increase in plasma potassium concentration directly increases K^+ secretion by the principal cells. Hyperkalemia also stimulates K^+ excretion indirectly by elevating plasma mineralocorticoid levels, which enhance K^+ secretion. When K^+ intake is decreased, K^+ excretion is decreased first as a result of decreased plasma aldosterone levels and later as a reduction of the permeability of the distal nephron for K^+ and decreased cellular K^+ content. Still another mechanism might be the increase of potassium reabsorption by the intercalated cells of the medullary collecting duct.
- Acid-base balance: metabolic acidosis inhibits K^+ excretion into the urine. Metabolic alkalosis on the other hand stimulates potassium secretion.
- Vasopressin: an increased Na^+ reabsorption and K^+ secretion is observed in the presence of vasopressin.

(Malnic et al., 1989; Fujii et al., 1990; Wade et al., 1990; Beck et al., 1992; Giebisch and Wang, 1996; Wang et al., 1997; Giebisch, 1998; Giebisch and Wang, 2000; Giebisch et al., 2000; Giebisch, 2001; Muto, 2001).

1.1.6 Concentrating of urine

One of the goals of the kidney function is to regulate extracellular water content of the organism in such a way that in situations of water overload, an excess of water is excreted and during water depletion, an excess of solutes is excreted in a minimal amount of water. Thus the kidneys must be able either to concentrate or to dilute the urine that is formed.

Besides the extracellular fluid volume, the kidneys also control the tonicity of this compartment as well as the regulation of systemic acid-base homeostasis, nitrogen and K^+ balance. To achieve simultaneous homeostasis, renal excretion of water, solutes and electrolytes, must be regulated independently from each other. Thus when water intake changes in the absence of changes in solute or electrolyte intake, the kidney must be able to excrete the appropriate amount of water without marked perturbations in solute excretion.

The ability of the kidney to concentrate and dilute the urine depends on a specialized organization of renal tubules and vasculature (Sands and Layton, 2000; Pallone et al., 2003).

The two populations of nephrons merge to form a common collecting duct system. Juxtamedullary nephrons have long loops that bend at various levels of the inner medulla. The cortical nephrons have loops that bend in the outer medulla. The limbs of Henle carry tubule fluid into and out of the renal medulla, establishing countercurrent flow between the two limbs of the loop. After exiting the loop of Henle, tubule fluid enters the distal convoluted tubule in the cortical labyrinth. Several distal tubules merge to form a connecting tubule arcade in most mammalian species. The collecting duct system spans all the regions of the kidney between cortex and papilla. They are arrayed parallel to the loop of Henle in the medulla and medullary rays and descend straight through these regions without joining other collecting ducts. Joining occurs in the inner medulla resulting in a progressive reduction in the number of inner medullary collecting ducts towards the renal papillary tip.

The descending and ascending vasa recta are the major blood vessels that carry blood into and out of the renal medulla. The descending vasa recta receive blood from the efferent arterioles of the juxtamedullary nephrons. They supply blood to the capillary plexuses at each level of the medulla, which subsequently enters the ascending vasa recta. Ascending vasa recta never receive blood directly from a descending vasa recta, preventing the shunting and increase of medullary blood flow (Masilamani et al., 2000).

The counterflow arrangement of vasa recta in the medulla promotes countercurrent exchange of solutes and water. The exchange is amplified by the presence of aquaporin 1 water channels and specialized urea transporters in the descending portion. Countercurrent

exchange provides a means of reducing the effective blood flow to the medulla, which is thought to maintain the interstitial concentrations of most solutes at levels close to those in the plasma in the vasa recta (Pallone, 2000; Pallone et al., 2000; Pallone et al., 2003).
A large amount of mucopolysaccharides is present in the inner medulla. These are thought to limit axial diffusion of solutes and flow of water in the inner medullary interstitium. Transport by the tubules can markedly alter the composition of the interstitial fluid owing to the function of the vasa recta, which maintain a low effective blood flow.
The cortical part of the renal interstitium is hardly affected by tubule transport because of the abundant blood flow in this region. As a consequence transport in the tubules in this region does not affect the transport in neighbouring tubules; in fact the rapid blood flow provides a very efficient buffer for the solutes and water absorbed and a large source for solutes that are being secreted (Masilamani et al., 2000).

The kidneys are able to both dilute and concentrate the urine. Hypotonicity of tubular fluid is primarily a result of active reabsorption of NaCl in the thick ascending limb of Henle. The osmotic permeability of water in these tubular segments is very low, thereby preventing dissipation of the transepithelial osmotic gradient by water fluxes. The hypotonicity of tubular fluid is maintained throughout the distal tubule and the collecting duct during water diuresis and is sustained by the low water permeability of the collecting ducts in the absence of vasopressin. Active NaCl reabsorption by the collecting ducts is mainly responsible for further dilution of the collecting duct fluid beyond that achieved in the thick ascending limb of Henle's loop, since the proportion of filtered water reabsorbed by the terminal collecting ducts is 1.6% during water diuresis, versus 0.6 % during antidiuresis (Jamison et al., 1973). The functional importance of this apparent contradiction will be explained later.

During antidiuresis, water is extensively reabsorbed between the distal tubule and the final urine. The major part being reabsorbed in cortical and outer medullary collecting duct, while the inner medullary collecting duct contributes only little to this process. The collecting duct fluid is concentrated by achievement of osmotic equilibrium between tubular fluid and the hypertonic medullary interstitium. The principal solutes responsible for the osmolality gradient in the medullary interstitium appear to be NaCl and urea (Sands and Layton, 2000).

Thus the process of urine concentration consists of two independent components. First the creation of a hypertonic medullary interstitium and second the achievement of the osmotic equilibrium of the tubular fluid in the collecting ducts with the hypertonic interstitium.
Generation of the interstitial hyperosmolality is largely dependent on the countercurrent multiplication system. It has mathematically been shown that a small concentration difference of maximum 200 mosm (called the single effect) between the ascending and descending limbs of a hairpin counterflow system can be multiplied by the countercurrent flow to obtain an axial gradient which is much larger than the transverse concentration difference between the limbs (Masilamani et al., 2000).
As an example we can predict what is happening in the descending limb. If there is a certain amount of fluid input at some point, then this fluid can be split into two fractions, a low diluted fraction following the transverse path into the interstitium and a concentrated fraction following the axial path.
If this process repeats at every subsequent segment, the system is able to produce a highly concentrated fluid at the end of the axis. One disadvantage of this system is that the total amount of fluid reaching the end (bend of the loop) becomes small (Masilamani et al., 2000).

It is now generally accepted that the single effect of this countercurrent multiplication system is generated by active NaCl reabsorption in the thick ascending limbs of Henle, at least in the outer medulla. Note that a basolateral Cl^- channel plays an important role in this process, as well as the apical NaK2Cl cotransporters. Of course the primary active Na-K exchanger must not be forgotten (Reeves and Andreoli, 2000). What really happens in the inner medulla depends on the characteristics of the thin limbs, which have little ability to actively transport NaCl. In theory, any process that would decrease the osmolality of the tubule fluid in the ascending limb to a level less than that of the surrounding interstitium could provide a single effect for concentration in the inner medulla. This could be achieved either by active transport or by passive transport using chemical potential energy from elsewhere in the kidney. Theoretically the last option can be achieved since the thin descending limb has a low permeability for NaCl, but a high permeability for water via aquaporins, AQP1. Water is reabsorbed along the thin descending limb. At the bend of the limbs, the NaCl is concentrated and thus has a chemical potential force to leave the lumen of the ascending part, especially if the permeability for water is low and urea has a lower

permeability compared to NaCl in this limb. This model can function as long as the efflux of NaCl is larger than the influx of urea. The values in table 1.3 indicate that these requirements are met in the rabbit kidney, i.e. a high permeability for NaCl, no permeability for water and only moderate permeability for urea. The transport of chloride appears to take place transcellularly through special chloride channels (ClC- K_1), while the sodium leaves this part via the paracellular pathway, driven by the electrochemical gradient that has been developed. The transepithelial potential difference is positive in the lumen. Absence of these chloride channels in mice impairs solute accumulation in the inner medulla, and leads to nephrogenic diabetes insipidus (Reeves and Andreoli, 2000; Uchida, 2000; Reeves et al., 2001; Akizuki et al., 2001).

Table 1.3: Permeability properties of thin ascending limb of Henle.

	$P_f(10^{-3}cm/s)$	$P_{Na}(10^{-5}cm/s)$	$P_{Cl}(10^{-5}cm/s)$	P_{Na}/P_{Cl}	$P_{urea}(10^{-5}cm/s)$
Rabbit	0	25.5	117	0.29	6.7
Rat	2.5	67.9	184	0.43	23.0
Hamster	3.0	87.6	196	0.47	18.5

P_f, osmotic water permeability, adapted from (Reeves et al., 2001).

In summary, a transepithelial osmolality difference of the appropriate orientation across any ascending or descending flowing stream in the inner medulla could provide a single effect for concentration of the inner medulla. However the initial process remains an active transport of NaCl out of the thick ascending limb of Henle, making the surrounding interstitium hyperosmotic. This hyperosmolality pulls water out of the thin descending limb of Henle. The single effect in this nephron segment is then started. Subsequent loss of water to the interstitium will concentrate the intraluminal fluid in the descending limb until the bend is reached. The exiting water cannot neutralise the interstitial hypertonicity as long as the ascending limbs keep transporting NaCl. This results in a highly concentrated intratubular fluid at the bend of the long limbs of Henle. NaCl at this point has obtained a large chemical driving force towards the interstitium, and leaves the thin ascending limbs of Henle in a passive way. Further in the thick part of this nephron segment, the NaCl is actively transported to the interstitium.

1.1.6.1 Role of urea

Urea is the major end product of protein catabolism in mammals. It makes up about 50% of urinary solutes in subjects consuming a “normal” protein diet. From a relatively low

concentration in plasma and glomerular ultrafiltrate urea can progressively be concentrated for about 100-fold along the nephron in the urine. Urea is usually considered to be concentrated indirectly with the support of several factors, including:

1. Osmotic energy provided by active NaCl reabsorption.
2. Influence of vasopressin (AVP) on water and urea permeation along the collecting ducts, which enables permanent delivery of concentrated urea in the deeper inner medulla.
3. Unique vascular-tubule relationships that reduce the dissipation of the intrarenal urea gradient by countercurrent exchanges and by intrarenal urea recycling.

Contrary to sodium accumulation in the kidney that depends on countercurrent multiplication in the loops of Henle, urea concentration requires the action of vasopressin in order to be concentrated in the medulla. Urea accumulation in the renal medulla is achieved through two associated processes: a permanent supply of concentrated urea to the medullary interstitium, which critically depends on AVP, and countercurrent exchanges between vasa recta and loops of Henle, which minimize the escape of urea in the venous blood draining the medulla. The latter is not affected by AVP. AVP increases the permeability to water along the whole length of the collecting duct, while urea permeability is only increased in the inner medullary part of this structure. In this way a urea gradient towards the interstitium is created in the medullary collecting duct, delivering concentrated urea to the medullary interstitium. This urea is continuously at risk for escaping the inner medulla, because the blood supplying the medulla readily takes it up. Due to the countercurrent action of the vasa recta this effect can be minimized, but it still depends on the rate of blood flow (Bankir and Trinh-Trang-Tan, 2000; Sands and Layton, 2000).

1.1.6.2 Achievement of urinary osmolality

The previous text has been dealing with the creation of the hypertonic medullary interstitium. In order to achieve the second part of the concentration process, i.e. the equilibration of tubular fluid osmolality with renal medullary interstitium, the transepithelial transport of water, NaCl and urea is important. In this case the aquaporins (AQP), the urea transporters (UT) and NaCl transport have to be mentioned.

Aquaporin-1 is very abundant in the proximal tubule and the thin descending limb of Henle, nephron segments that are exceedingly permeable to water, and completely absent in those segments with virtually no water permeability, thick ascending limb of Henle and distal

tubule. The critical role of AQP1 in urinary concentration was shown in studies using AQP1 knockout mice. These animals were polyuric and had a reduced urinary concentrating capacity. Moreover it was shown that there was an 80 – 90% reduction in osmotic water permeability in the proximal tubule and descending limb of Henle (Vallon et al., 2000). AQP1 seems to be the principal water channel in the descending limb of Henle and this supports the view that osmotic equilibration along this nephron segment by water transport plays a key role in the renal countercurrent concentrating mechanism. It is essential for the single effect of countercurrent amplification in the loops of Henle (Chou et al., 1999b; Agre et al., 2000; Kwon et al., 2001).
Moreover the AQP1 channels have also been demonstrated in descending vasa recta and therefore may account for the maintenance of the medullary osmotic gradient (Pallone et al., 2000).

AQP2 is the apical water channel of collecting duct principal cells. Vasopressin mediated trafficking of AQP2 represents the cellular mechanism underlying the acute regulation of body water balance by vasopressin (DiGiovanni et al., 1994; Nielsen et al., 1995; Ward et al., 1999; Brown and Nielsen, 2000; Nielsen et al., 2000).
The water permeability of the collecting duct and the urinary concentration as such, also undergo adaptational regulation. This response is associated with changes in the total number of AQP2 water channels per cell. Water restriction or chronic vasopressin treatment increases AQP2 expression and collecting duct water permeability, whereas water loading or treatment with vasopressin antagonists decreases these parameters (Kwon et al., 2001). Fasting seems to downregulate the AQP2, leading to polyuria (Amlal et al., 2001).
AQP3 and AQP4 have been shown to be abundantly expressed in the basolateral plasma membrane of collecting duct principal cells. Possibly they function as exit pathways for the water reabsorbed on the apical side via AQP2. AQP3 is more distributed along the cortical and outer medullary part of the collecting duct, whereas AQP4 is more abundant in the inner medullary part. Other aquaporins most probably play a minor role in the kidney.
Dysregulation of AQP2 and to a lesser extent AQP3 plays a major role in water balance disorders or conditions with altered urinary concentration. Among these inherited and acquired nephrogenic diabetes can be mentioned (Ma et al., 2000; Kwon et al., 2001).
However, AQP4 absence does not influence renal concentrating ability (Chou et al., 1998a; Ma et al., 2000).

Urea transporters can generally be grouped into transporters expressed in the kidney (UT-A) and in red blood cells (UT-B). These transporters are responsible for the facilitated diffusion of urea through cellular membranes. The UT-A receptors have been subdivided into four groups, of which the UT-A1 is regulated by AVP (Sands, 1999). Besides this rather fast effect of vasopressin (upregulation), it has been demonstrated in Brattleboro rats (neurogenic diabetes insipidus) that there is also a long term regulation of AVP on both UT-A1 and UT-A2 transporters, the first one being downregulated and the second one being upregulated (Shayakul et al., 2000).

The UT-B transporter in erythrocytes permits these cells to lose urea rapidly as they traverse the ascending vasa recta, thereby preventing loss of urea from the medulla and decreasing urine-concentrating ability by decreasing the efficiency of countercurrent exchange. Besides these passive transporters, secondary active transport mechanisms have also been characterized in inner medullary collecting duct subsegments. These can be expressed under special conditions, such as hypercalcemia, low protein diet and under the influence of furosemide (Kato and Sands, 1999; Sands, 1999; Bankir and Trinh-Trang-Tan, 2000).

Water excretion is regulated by vasopressin, largely as a result of its effect on the water permeability of the collecting ducts. When the water permeability is low in collecting ducts because of a low circulating level of vasopressin, relatively little water is reabsorbed. The dilute fluid exiting the loops of Henle remains dilute as it passes along the collecting duct system, resulting in a large volume of diluted urine. A high circulating level of AVP increases the permeability of the collecting duct and water is osmotically extracted. The osmolality of the final urine will approach that of the inner medullary interstitium. The amount of water, absorbed in the initial cortical collecting duct, required to raise tubule fluid to isotonicity is considerably greater than the additional amount required to concentrate the urine to greater than plasma osmolality in the medullary portion of the collecting duct system. In this way, most of the reabsorbed water enters the renal cortex, where blood flow is high enough to return this water to the general circulation without the risk of diluting the interstitium. During water diuresis more water is reabsorbed from the terminal collecting ducts than during antidiuresis, owing to a much larger transepithelial osmolality gradient. This high rate of water reabsorption is thought to contribute to the reduction of medullary interstitial osmolality during water diuresis by its dilutional effect.

Aside from its effects on water permeability in the collecting duct system, vasopressin has additional effects that are important to the overall function of the concentrating mechanism. As has already been mentioned, AVP increases the urea permeability in the terminal part of the inner medullary collecting duct and thus regulates the delivery of this solute to the medullary interstitium (Sands, 1999a; Masilamani et al., 2000). AVP increases the rate of NaCl absorption in the thick ascending limb of the loops of Henle, thereby enhancing countercurrent multiplication, which is important in the generation of a hypertonic interstitium. Probably it also increases the rate of NaCl reabsorption in distal tubule and collecting duct (Elalouf et al., 1984; Masilamani et al., 2000). This effect might help in shifting water reabsorption to the cortex during antidiuresis, instead of loading the medulla and diluting the interstitial fluid.

Up to this point it has been emphasized that medullary interstitial osmolality is mainly built up of NaCl and urea. However, there are a number of other solutes that also accumulate in the medulla and contribute to overall osmolality. Besides NaCl and urea, NH_4^+ and K^+ appear to be the major components in the medullary interstitium. The high interstitial medullary osmolality must be matched by an equally high intracellular osmolality. The renal cells could compensate this osmolality by an equal rise in intracellular NaCl and urea, but these substances are detrimental to cell function because they affect many vital functions of the intracellular macromolecules. The cells in the renal medulla minimize these effects by increasing the intracellular organic solute content. Several different organic solutes accumulate in renal medulla cells in substantial concentrations, i.e. sorbitol, inositol, glycerophosphocholine, betaine and taurine. The accumulation of renal organic osmolytes by net synthesis or transport is a relatively slow process that requires hours to days. However, when these cells are suddenly exposed to a rather isosmotic environment, they are able to rapidly lose the organic osmolytes, probably via specialized transporters (Blumenfeld et al., 1989; Nakanishi et al., 1991; Burger-Kentischer et al., 1999; Beck et al., 2000; Masilamani et al., 2000; Grunewald et al., 2001).
Furthermore, the structure of the renal interstitium changes in response to AVP. The total amount of osmolytes increases in the form of hyaluronic acid, stimulating water reabsorption even more (Law and Rowen, 1981; Rowen and Law, 1981; Rowen and Law, 1981).

1.2 Isolated perfused kidneys

Thus, the kidney is a highly complex organ that functions in a highly coordinated fashion and it needs no further argumentation that renal function under health and disease states has been extensively investigated. To understand disease states based on kidney dysfunction, a fair knowledge of kidney physiology and pathophysiology is required. Understanding these matters may lead to a more efficient and effective (pharmacological) treatment of kidney diseases and disease states.

The interdependence of organs makes that function of the organism cannot be reduced to a simple utilisation of animal, organ, tissue and cell culture models. However these models are essential in obtaining the data necessary to understand normal and abnormal renal function but one must keep in mind that every model has its strengths and weaknesses. It is clear that certain renal functions as glomerular filtration dynamics, cannot be investigated with tissue or cell cultures (Lieberthal and Nigam, 2000).

The interdependence of organs also requires that the experimental study of renal function be dissociated from extrarenal and possible intrarenal control mechanisms. One of the principal reasons to develop and improve isolated kidneys was that studies in vivo are often complicated by concurrent changes in blood pressure, circulating fluid volume, CO_2 tension or neurogenic and hormonal responses to the experimental stimulus (Ross, 1978). For instance, infusion of an angiotensin converting enzyme (ACE) inhibitor will result in a widespread vasodilatation leading to a decrease of arterial blood pressure. This decrease in turn will stimulate the production of renin and angiotensin II and in a way counteract the inhibitory effect of the ACE antagonist.

The isolated kidney is halfway the spectrum of strengths and weaknesses. Moreover, it is also dissociated from systemic alterations. The first degree of isolated kidney perfusion corresponds to the denervated in situ operating organ, in which the nervous influences on renal function are supposed to be eliminated. The next degree of isolated perfusion is the use of the animal's heart as perfusing pump and the lungs as oxygenator. The completely isolated perfused kidney is achieved with perfusion by an artificial heart and lung (Nizet, 1975).

On the other hand, the isolated kidney can be subjected to a wide range of variables, which would not be allowed by an in vivo model. For instance, the urea or oxygen delivery to the

kidney can be reduced to almost zero, without eliciting compensatory responses in other organ systems. A situation that is not imaginable in vivo. This is also true for investigation of renal handling of drugs (Bekersky, 1983b).

1.2.1 History of isolated organ perfusion

The idea to artificially perfuse organs had already originated in the 18th century, when JJC Le Gallois thought of a substitute for the heart to maintain any part of the body indefinitely, but the first attempts to perfuse isolated organs were made in the 19th century by Löbell. In 1885, Max von Frey designed an apparatus that had characteristics of a heart – lung machine. With this device, he perfused an entire lower extremity of dogs and took measurements of oxygen consumption and carbon dioxide and lactate production. The artificial lung consisted of a hermetically closed glass cylinder, with a centrally fixed metal core. The glass cylinder was constantly rotating around a slightly oblique horizontal axis so that the venous blood that had entered at the top of the cylinder adhered to the inner wall. The blood was collected at the bottom. It showed slight similarity with the apparatus used in the present study (chapter on materials and methods).

In 1935 Charles A. Lindbergh together with Alexis Carrel developed a pulsating device, which could be used to perfuse organs like thyroid, ovary, adrenal gland, spleen, heart and kidney of fowls and cats. The apparatus consisted of two systems; the perfusion line and the pulsating gas pressure system. For these two systems to function, three glass chambers were placed in a vertical line on top of each other. The upper chamber was the organ chamber and the lower chamber was the fluid reservoir. The artery of an organ was mounted on and perfused with an artificial medium by a cannula.

In 1937 John H. Gibbon had developed and tested a closed circuit heart-lung machine to institute cardiopulmonary bypass in cats during experimental occlusion of the pulmonary artery and in 1953 he had performed the first successful open-heart surgery in a patient using a heart-lung machine. The first physiologist to perform systematic and quantitative studies of isolated organs was Carl Ludwig of the institute of Physiology in Leipzig, Germany. The fact that organs can be isolated and kept functioning for a considerable period of time by perfusing them with blood or an artificial medium was essential for the idea of organ transplantation. Alexis Carrel developed a technique to suture vessels and started to transplant organs in animals in the early 20th century. In the course of the

experiments on isolated, perfused organs it became clear that the oxygen and carbon dioxide content and metabolic products in the perfusing medium should be measured.

Perfusion of isolated organs became a widespread application in physiology and included the heart, pancreas, muscle, lung, liver and kidney. Gradually it appeared that beside the investigation of renal function, isolated perfusion of kidneys could also contribute to better performance of renal transplantation. With the introduction of the isolated perfused rat kidney by Weiss et al in 1959, the era of isolated perfused kidneys started (Weiss et al., 1959; Ross, 1978; Nizet, 1978; Zarzuelo et al., 2000; Zimmer, 2001).
In all those years, kidneys of various animals have been isolated and perfused. Amongst these the isolated rat kidneys far outnumber the others, which include dog, rabbit, pig, sheep, monkey and even human kidneys (Nizet, 1975). The convenient size, genetic uniformity and low cost are factors in favour of the rat (Ross, 1978). Not only did the isolated perfused kidney prove to be a valuable tool in the research of renal physiology and pathophysiology (Sumpio and Maack, 1982; Kau and Maack, 1986), it also became a widespread application in pharmacology in recent years. Isolated kidneys are used as experimental model in drug clearance studies and pharmacokinetic studies (Bekersky, 1983a; Bekersky, 1983b; Zarzuelo et al., 2000).

In spite of the numerous efforts to study renal function under well-defined conditions, most of the isolated kidneys were restricted with regard to their functional abilities. Essential functions like the glomerular filtration rate and net tubular sodium reabsorption, renal perfusion flow and the ability to concentrate were more or less impaired (Franke et al., 1971).
In the early years, most of the perfusions were attempted with blood, but in most cases some degree of vasoconstriction made the functional performance of the kidney poor. Improvement of renal function was achieved with a perfusion circuit including the heart, lung and kidney. Some investigators added various drugs to the blood. Eventually Nizet and Cuypers (Nizet et al., 1967; Nizet, 1978) proposed the following conditions, which were based on investigations performed on an isolated dog kidney, to achieve an optimal function with good perfusion:

- The perfusion should start within a maximum delay of 3 minutes after blood has been withdrawn from the donor animal, with the addition of a short acting vasodilating agent.
- Mechanical stirring of the blood should be avoided.
- Blood volume should not exceed 15 times the weight of the kidney, allowing the organ to sufficiently eliminate possible vasoconstricting factors.

Brandani Pacini and Bocci, although taking these precautions in consideration, still encountered the vasoconstriction with whole blood. They found that the use of platelet and leucocyte poor blood improved the functional parameters (Brandani Pacini and Bocci, 1983).

The other, somewhat easier solution for the problem of vasoconstriction was the use of artificial cell free perfusion liquids such as buffered saline. In fact most investigators chose for this option (Nizet, 1975). Numerous attempts have been made to prepare a suitable perfusion solution. Very soon it became clear that a simple buffered saline did not meet the requirements. There were a lot of functional and structural defects: edema formation, low GFR, low Na^+ and water reabsorption, low renal perfusate flow and little or no urine concentrating ability. The first attempts to correct these difficulties were aimed at the creation of colloid osmotic pressure in the perfusing fluid. The addition of albumin to the regular saline improved renal function. Several colloid osmotical agents have been tried out in varying concentrations. Based on a comparative study, Ross concluded that the bovine serum albumin (BSA) fraction V in a concentration of 7.5% - 8% w/v, was the most satisfactory one (Ross, 1978). Little and Cohen (Little and Cohen, 1974) perfused rat kidneys with a bicarbonate-buffered physiological saline with varying concentrations of BSA fraction V (0 – 8%). They found that increasing the albumin content of the perfusate led to:

- A decreased kidney water content to almost control values.
- An increased perfusate flow rate from 5 $ml \bullet min^{-1} \bullet g^{-1}$ to 21 $ml \bullet min^{-1} \bullet g^{-1}$, which was attributed to a reduction in the volumes of the interstitial and lateral intercellular spaces, i.e. reduction of edema.
- An increased fractional Na^+ reabsorption from 55% to 95%, which was explained by the increase in peritubular vascular colloid osmotic pressure.

The mean GFR remained lower than normal and was not affected by the increase in BSA content (Little and Cohen, 1974). On the other hand, Schurek and Alt found that increasing

albumin concentration decreased the GFR significantly (Schurek and Alt, 1981). Franke et al achieved an improved net reabsorption of sodium and higher GFR with the use of a non-ionic detergent (Pluronic-F-108) as colloid osmotically active substance (Franke et al., 1971).

It became clear that the use of colloid osmotical agents in the perfusing solution, improved renal function significantly with regard to the reabsorptive functions: excessive natriuresis and diuresis could be prevented (Maack, 1980; Swanson et al., 1981).
In order to improve tubular function of the isolated kidney preparations the addition of specific substrates for renal metabolism besides glucose was tried out. When a substrate enriched solution, containing pyruvate, lactate, oxaloacetate and glutamate, was used sodium conservation of the isolated kidney could be maintained at a higher level (Schurek et al., 1975). The addition of amino acids to the perfusate further improved renal function and was one of the important achievements in developing a suitable perfusate for isolated perfusion (De Mello and Maack, 1976; Maack, 1980). Epstein et al demonstrated that addition of 20 physiological amino acids to the perfusate, prevented some of the anatomical defects, observed in their absence and kept GFR and sodium reabsorption close to the initial value during the four hours of perfusion (Alcorn et al., 1981; Epstein et al., 1982).

1.2.2 Choice of perfusate

An appropriate composition of the artificial perfusate is of critical importance for a stable functioning isolated kidney. Several compositions have been proposed, but the most widely used is Krebs-Heinseleit bicarbonate buffer containing glucose as the metabolic substrate and a macromolecule as an oncotic agent. The use of BSA fraction V as colloid osmotic active substance is preferred, because of the deleterious effects of synthetic polymers (Maack, 1980). Table 4 shows the approximate composition of Krebs-Heinseleit and Tyrode's solution compared to plasma values of rabbit. Depending on the purpose of the experiments to be performed, other substances are added. Basically it consists of the electrolytes in table 4, with the addition of glucose, BSA, urea and amino acids. Some authors prefer the addition of inulin, while others use creatinine or polyfructosan in order to estimate the GFR. In spite of all the modifications, some structural and functional defects remained in the isolated preparations. One important defect was recognised by Alcorn et al (Alcorn et al.,

1981). During perfusion, even under optimal conditions, consistent and reproducible changes indicated that cellular necrosis developed in the cells of the thick ascending limb of Henle. Severity of the injuries was proportional to the duration of perfusion and distance to the blood vessels and the necrosis was specifically located in the outer medullary region (Alcorn et al., 1981; Schurek and Kriz, 1985).

Table 1.4: Composition of two widely used solutions for the perfusion of isolated kidneys.

Perfusate / Composition	Krebs-Heinseleit[1]	Tyrode[2]	Rabbit Plasma[3]
Na^+ (mM)	140	150	143
K^+ (mM)	5	3	3.6
Ca^{2+} (mM)	2.5	1.8	3.4
Mg^{2+} (mM)	1.2	1.1	0.5[4]
Cl^- (mM)	117	145	100
HCO_3^- (mM)	27	12	13.3
HPO_4^{2-} (mM)	1	0.5	1.9

Brezis at al proposed that these structural defects might be the result of the transport activity of the epithelial cells of the thick ascending limb of Henle. These cells actively transport NaCl and have a high metabolic requirement (Brezis et al., 1984). On the other hand, their oxygen supply becomes limited because of the countercurrent arrangement of the vasa recta and hence the shunt effect between descending and ascending vessels (Schurek and Kriz, 1985; Pallone et al., 1998; Pallone, 2000). Taken into account that oxygen transport by red blood cells is substantially higher than with plasma alone (Hall and Guyton, 2000), it is predictable that the oxygen delivery to these specific cells by low hematocrit perfusate does not meet their demand resulting in morphological and functional defects, with respect to concentrating and diluting ability. In addition, the unphysiological high perfusion rate observed with artificial cell free media that might be ascribed to the very low viscosity washes out the osmotic gradient in the medulla and contributes to the deterioration of the concentrating ability (Masilamani et al., 2000).

[1] (De Mello and Maack, 1976; Ross, 1978; Maack, 1980)
[2] (Bagate et al., 1999; Bagate et al., 2000; Bagate et al., 2001)
[3] (Cuypers et al., 2000)
[4] (Kozma et al., 1974)

Table 1.5: Renal parameters in the presence of different amounts of erythrocytes, as demonstrated by Lieberthal and co-workers in isolated perfused rat kidneys (Lieberthal et al., 1987).

Hematocrit / Parameters	0	4 – 6%	20 – 25%	40 – 45%
RPF (ml/gkw.min)	25±1.1	25.3±1.2	14.3±1.2	9.1±0.4
GFR (ml/gkw.min)	0.83±0.05	0.93±0.03	0.60±0.03	0.57±0.04
FF (%)	3.3±0.3	3.9±0.3	5.4±0.5	10.8±0.6
FeNa (%)	14.5±1	9.4±0.3	3.9±0.7	3.5±0.6
Uosm (mOsm/kg)	343±4	366±39	-	640±35
mTAL necrosis	++	-	-	-

RPF, renal perfusate flow, GFR, glomerular filtration rate, FF, filtration fraction, FeNa, fractional excretion of sodium, Uosm, urinary osmolality, mTAL, medullary thick ascending limb of Henle.

Table 1.6: Overview of differences between blood perfused and artificial fluid perfused kidneys of various species.

	Blood	Artificial
RPF	(Initial) vasoconstriction	Unphysiologically high
GFR	Low	Low - normal
Filtration Fraction	Normal	Very low
Na reabsorption	Low-Normal	Very Low
Diluting/concentrating capacity	Present/almost normal	Poor/very low
Technique	Difficult/complex	Easy/simple
Autoregulation of GFR/RPF	Incomplete	Absent
mTAL necrosis	Minimal/Absent	Abundant

RPF, renal perfusate flow, GFR, glomerular filtration rate. Based on (Nizet et al., 1967; Bowman and Maack, 1972; Little and Cohen, 1974; Nizet, 1975; Schurek et al., 1975; De Mello and Maack, 1976; Ross, 1978; Bullivant, 1978; Nizet, 1978; Maack, 1980; Swanson et al., 1981; Alcorn et al., 1981; Epstein et al., 1982; Brandani Pacini and Bocci, 1983; Schurek and Kriz, 1985; Lieberthal et al., 1987; Heringlake et al., 1999; Cuypers et al., 2000; Arnaud et al., 2000).

The absence of urine concentrating ability is so common, that only few investigators mention urine osmolality (Nizet et al., 1967; Nizet, 1975; Brandani Pacini and Bocci, 1983; Lieberthal et al., 1987).

Lieberthal et al., (1987) nicely demonstrated the need for the presence of erythrocytes in the perfusate. They perfused rat kidneys with Krebs-Heinseleit buffer in the presence of varying amounts of bovine red blood cells. Results are summarised in table 5. Besides the improvement in filtration fraction and urine osmolality, the presence of erythrocytes also

led to an improvement of autoregulation of renal perfusate flow and GFR (Lieberthal et al., 1987). Indeed, most of the kidneys perfused with artificial fluid do not demonstrate autoregulation of GFR, neither renal perfusate flow (Bullivant, 1978; Gleim et al., 1984; Heringlake et al., 1999). Swanson and coworkers also accentuate the importance of erythrocytes for the reabsorption of sodium (Swanson et al., 1981).

Nevertheless, the use of artificial perfusion solutions has been accepted and is widely used. Table 6 illustrates the differences in functional performance of isolated kidneys perfused with blood versus artificial perfusate. The choice of perfusion medium will depend on the purpose of the experiments. For instance, if the investigation is aimed at studying the concentrating ability of the kidney or the function of the thick ascending limb of Henle, then it would be better to use blood as perfusion medium (Lieberthal et al., 1987). In contrast investigation of the filter properties of the glomerulus would better be performed with the use of artificial media (Ciarimboli et al., 1999).

1.2.3 Modes of perfusion

Besides the arrangement of blood-perfused and artificial fluid-perfused kidneys, there is a difference in approach with respect to the mode of perfusion. First there is the approach of single pass through the isolated perfused kidney. In this preparation, the fluid enters the kidney on the arterial side and after leaving the kidney at the venous side it is discarded. This system is only useful while applying artificial solutions. It is employed when a constancy of input or functional parameters is required. One disadvantage is the costs of the albumin used in this system.

The second approach is the recirculatory system (Schurek, 1980). In contrast to the single pass system, blood or albumin containing perfusate can be used without increasing costs in this system. A disadvantage is that functional parameters decline in quality, probably caused by growth of bacteria (Hems and Gaja, 1972; Schurek, 1980; Bagate et al., 1999; Kurtz et al., 2000; Bagate et al., 2000; Bagate et al., 2001). The advantages of the two systems can be combined in the recirculatory system with dialysis according to Schurek, (1980).

Still another mode of perfusion is the isolated non-filtering kidney. In this preparation the albumin content is increased to a value that results in an oncotic pressure, which prevents

ultrafiltration. These preparations are used to study the extraction and metabolism of peptide hormones at the peritubular side of the kidney (Maack, 1980).

Finally there are the isolated perfused hydronephrotic kidneys. These are used to investigate the activity and reactivity of the renal microvasculature. In these kidneys, the hydronephrosis is caused in the animal through a six week ligation of one ureter. In the kidney the major part of the medulla and part of the cortex atrophy, leaving a small line of cortical tissue. In this cortical part it is easy to visualize glomeruli and other vascular structures. The hydronephrotic kidney is thereafter isolated and perfused (Nishikawa et al., 1977; Takenaka et al., 1998; Tang et al., 1999; Takenaka et al., 2003). The application of split hydronephrotic kidneys in vivo was not uncommon (Steinhausen et al., 1981; Nobiling et al., 1986; Steinhausen et al., 1989).

Arnaud et al have reported data on perfused rabbit kidneys (see table 1.7) with homologous blood recently, whereas Cairns et al have used a cell free medium to perfuse rabbit kidneys (Cairns et al., 1989; Arnaud et al., 1998, 2000). There is an older report by Brandani Pacini and Bocci, but the report is presented in a rather indecipherable way (Brandani Pacini and Bocci, 1983).
An overview of different studies using isolated kidney preparations is given in table 7 at the end of this chapter. The number of recent publications using the isolated kidney preparations demonstrates the usefulness of this method (Fonteles et al., 1998; Heringlake et al., 1999; Garcia et al., 1999; Santos-Neto et al., 1999; Nobre et al., 1999; Monteiro et al., 1999; Zarzuelo et al., 2000; Monteiro et al., 2001; Van der Giet et al., 2001; Gui et al., 2003). However it is obvious that the fractional reabsorption of Na^+ does not reach 99% and urinary osmolality is not mentioned in most cases.

Table 1.7: Overview of results obtained with different isolated kidney preparations.

animal	Perfusion medium	RPP (mmHg)	RPF (ml/gkw.min)	GFR (ml/gkw.min)	Uv (µl/gkw.min)	FR_{Na+} (%)	$FR_{glucose}$ (%)	U osm (mOsm/kg)	System	Reference
Rat	KH + BSA	100	17-20	0.8 – 1.0	103 - 108	89	95	-	Recirculatory[1]	(Radermacher et al., 1999)
Rat	KH + BSA	100	30	0.56	-			-	Recirculatory	(Lebowitz et al., 1992)
Rat	KH + BSA	100	30	0.7 – 0.9	-	-	-	-	Recirculatory	(Perico et al., 1997)
Rat	KH + BSA	105±5	19	0.84	63	-	-	-	Single pass	(Murray and Churchill, 1984)
Rat	KH + BSA[2]	90 - 100	-	0.81	66±24	94.7	-	398±10	Recirculatory	(Epstein et al., 1982)
Rat	KH + BSA	90	50	0.71	40	97.5	-	-	Recirculatory	(De Mello and Maack, 1976)
Rat	KH + BSA + RBC	90 - 100	21	0.76	77±29	94±2	96±1	-	Recirculatory	(Schurek and Kriz, 1985)
Rat	KH[3]	90 - 120	14	1.0	300 - 500	72	97	-	?	(Franke et al., 1971)
Rat	KH	100	15.6	1.38	683	51.3	78.5	-	Recirculatory	(Schurek and Alt, 1981)
	KH + Albumin		36.4	0.56	30	97.1	94.5	-		
Rabbit	blood[4]	87±5	3.7±1	0.29	110	67.9±8.5	91.2±5.8	-	Recirculatory[5]	(Arnaud et al., 1998, 2000)
Rabbit	KH + NE	90	22.3/kidney	2.04/kidney	-	-	-	-	Single pass	(Cairns et al., 1989)
Dog	Blood[6]	110	3.8	0.34 – 0.53	< 35			500 – 800	Recirculatory	(Nizet et al., 1967)
Pig	Blood[7] (hct 0.33)	96.9±16.9	1.24±0.59	0.099	24	-	-	-	Recirculatory With Dialysis	(Dittrich et al., 2000)
	Blood (hct 0.21)	83.8±15.0	1.34±0.52	0.156	57	-	-	-		
Pig	KH + BSA	100	8.5	0.65	2 ml/kidney	39 - 46	-	-	Recirculatory	(Goujon et al., 1999; Hauet et al., 2000)

RPP, renal perfusion pressure; RPF, renal perfusate flow; Uv, urine flow; FR, fractional reabsorption; osm, osmolality; KH, Krebs-Heinseleit; BSA, bovine serum albumin fraction V; NE, norepinephrin; RBC, red blood cells.

[1] Diclofenac used to inhibit effects of prostaglandins
[2] No amino acids added
[3] Artificial oncotic agent
[4] diluted to hematocrit 25%, homologous
[5] high degree of hemolysis
[6] Heparinized, autologous
[7] autologous

Chapter 2: Aim of the study

Aim of the study

The purpose of this study is to characterize whether the isolated rabbit kidney is a suitable model to investigate renal physiology, pathophysiology and pharmacology. The isolated kidney of the rabbit was chosen in order to bypass systemic effects on the kidney. It was perfused with autologous blood. Moreover, it was investigated whether this preparation is suitable for micropuncture studies.

In chapter 3 a general description is given of the materials and methods used in this study. Culprits are mentioned and directions to perfuse such kidneys are given. The preparation is characterized in chapter 4. Renal blood flow (RBF), glomerular filtration rate (GFR) and the factors that are generally regulated through tubular function (glucose, phosphate, sodium, water and potassium excretion and concentrating capacity) are determined. These parameters are measured under different conditions, such as variations in perfusion pressure as well as the presence or absence of the antidiuretic hormone (arginine vasopressin) and the presence or absence of urea in a compensating infusion. An attempt is made to compare the determined parameters with reported values found in vivo as well as in vitro studies.

Since adenosine is proposed as the mediator of the tubuloglomerular feedback mechanism (Schnermann and Levine, 2003), which might be involved in the pathophysiology of acute renal failure after renal ischemia, chapter 5 deals with the effects of adenosine and the adenosine blockers, DPCPX (A_1 adenosine receptor antagonist) and DMPX (A_2 adenosine receptor antagonist), on the function of this preparation.

In chapter 6 an investigation of the paracrine influences of NO on the microcirculation and the tubular function of this preparation was attempted.

Chapter 7 reports the effects of an osmotic diuretic (mannitol) and a loop diuretic (furosemide) on glomerular and tubular function.

Finally, in chapter 8 micropuncture studies are performed on this isolated perfused kidney. The free flow hydrostatic pressure is measured in proximal tubules in control conditions, under hypoxic circumstances and when hypertonic mannitol is infused in the presence or absence of hypoxia. The findings are compared with available data in the literature.

Chapter 3: Materials and methods[1]

[1]Part of this chapter has been published in: Cuypers, Y., Vandenreyt, I., **Bipat, R.**, Toelsie, J., Van Damme, B., and Steels, P. The functional state of the isolated rabbit kidney perfused with autologous blood. *Pflügers Arch* 440: (634-642) 2000.

Materials and methods

3.1 Kidney preparation

In these experiments white rabbits weighing 3 – 3.8 kg were used as kidney donors. The rabbits were kept in metabolic cages. Animals had free access to tap water and were fed commercial pellets (protein: 17.6%, fat: 4%, fibres: 16%). In the twenty-four hours preceding the experiment, they were not exposed to excessive stress. Preanaesthesia was induced with 0.7 ml of Thalamonal® (Janssen Pharmaceutica, 2.5 mg/ml droperidol + 0.05 mg/ml fentanyl) intravenously. Anaesthesia was carried out with 0.6 ml of ketamine (50mg/ml) and sustained via a vein in the ear. NaCl 0.9% was infused at a rate of 6 ml/hour in the ear vein. Under anaesthesia, the trachea was prepared free and a polyethylene tube was inserted after tracheotomy. Artificial respiration (Bird) was started immediately. Following this procedure the left carotid artery was isolated. A heparinised polyethylene catheter (Clay –Adams, PE 190, ID 1.17 mm, OD 1.7 mm) was inserted and processed to the aorta. Blood pressure was monitored constantly via this line with a Statham ID 23 sensor and recorded digitally via a Powerlab 800/s (Adinstruments, Australia) on a personal computer. Meanwhile the animal was kept under anaesthesia.

At this point the animal body was turned and the left flank was exposed and shaved. An almost longitudinal incision was made paravertebrally. Small blood vessels were thermally coagulated; larger ones were ligated. The kidney was approached through the retroperitoneal space. Intestines were kept as far as possible in the intraperitoneal cavity, however they sometimes protruded through the peritoneum.
Perirenal fat was cautiously, but maximally removed from the kidney surface, since this appeared to be an important source of blood loss during perfusion. The kidney was not touched unnecessarily. This procedure should prevent macroscopic hematuria. After the ureter and renal vessels had been exposed, papaverine (50 mg/ml) was applied to their external surface. This was done as a precaution to extensive vasoconstriction during manipulation. When the kidney was completely exposed, 0.5 ml xylocaine (10 mg/ml) was applied on the vessels, in order to prevent vascular spasm or retraction after extirpation.

The kidney was then reinserted in the abdomen. Meanwhile the arterial and venous catheters had been prepared. Heparin (5000 IU in 5 ml NaCl 0.9 % solution) was injected

via the carotid artery in the systemic circulation. Hereafter a small amount of the heparinised blood (4 - 5 ml) was withdrawn and injected in the artificial heart-lung machine (figure 3.7) and recirculated together with saline. This fluid was completely removed from the artificial perfusion system after the rotating cylinder in the gas exchange chamber was completely coated. After coating of the circulating device, the kidney was gently pulled out of the abdomen, without unnecessary touching again. The ureter was first thermally cut with the coagulator. The vascular tree was clamped and the vessels were cut off proximally. Within 2 - 3 minutes the artery and vein were catheterised (artery: PE 190, ID 1.17, OD 1.7 mm filled with 9 g/l NaCl solution; vein: silicone, ID 2.0 mm, OD 4.0 mm) and the organ was transferred and connected to the artificial pump and oxygenator. The then isolated kidney was put on a support that was kept at a temperature of 38 °C (figure 3.8). Meanwhile an amount of about 25 ml of blood was extracted from the animal and very gently injected into the perfusion system (figure 3.7). Care had to be taken here, because foaming of the blood might cause an extensive vasoconstriction. Pumping via a circulation system bypassing the kidney was immediately started to remove air bubbles from the "arterial" part of the system. As soon as this was achieved, the bypass was disconnected and circulation through the kidney was started. The whole procedure in most instances did not take more than 5 minutes. Mean perfusion pressure was constantly kept within a narrow range of 100 mmHg; blood flow was continuously monitored and recorded digitally via a Powerlab 800/s.

The kidney was superfused by warm paraffin oil (38 °C).
Immediately after circulation through the kidney had been secured, catheterisation of the ureter was started (PE 205, ID 1.57, OD 2.08). Needless to say that this had to be done gently in order to prevent macroscopic hematuria again (Cuypers et al., 2000).
In order to obtain a preparation with a fairly good concentrating capacity, blood was used instead of artificial perfusate. It has also been demonstrated that the presence of red blood cells in the perfusate prevents damage to the mTAL in contrast to kidneys perfused with cell-free media (Alcorn et al., 1981; Lieberthal, 1991). In addition, as can be seen in table 1.4, the electrolyte composition of plasma differs from the regular solutions used. No attempt was made to remove components of the blood, since it is the purpose that in ischemia-reperfusion studies, the different components of blood and kidney are allowed to

react in a normal physiological way under presumed normal physiological conditions. However, clotting is prevented with heparin.

The precautions as described by Nizet (Nizet et al., 1967; Nizet, 1975, 1978) and mentioned earlier in the text, were taken into account except that the use of short acting vasodilators was omitted in the present study.
Since there had to be a minimal volume of blood in the oxygenator in order to prevent gas aspiration, the minimal size of the kidney had to be larger than the conventional rat kidney. That is one of the reasons why the rabbit kidney was chosen.
The use of heparinised blood made the application of a recirculatory system obligatory, as has been explained earlier in the text.
With the above mentioned precautions and considerations, the perfusion of isolated rabbit kidneys with autologous blood was undertaken.

3.2 Circulating device and perfusion procedure

The circulating device consists of a pulsating pump and an oxygenator (figure 3.2 and 3.7). The pump is driven by a pulse-generating device (figure 3.1). The piston generates pulses, which are transmitted through water in a closed silicone tubing system. These pulses repeatedly compress a latex tube of about 25 cm length situating in a closed Plexiglas chamber. At both ends of the latex tubes, ball valves direct the blood stream in only one way to the kidney. The rate of pulses is constant at 163 rpm.

The amplitude of the stroke in the circulating device is damped by use of a syringe filled partly with blood and partly with air. Figure 3.6 demonstrates the effect of the damping system on the perfusion pressure.
Damping of the pulse pressure is necessary, especially when one wants to perform micropuncture studies on the kidney. Moreover great variations in perfusion pressure were prevented and a more physiological profile was obtained. The beneficial effect of damping is illustrated in figure 3.6.
Blood flow can be varied by changing the amount of blood pumped per pulse. This is achieved by changing the amplitude of the piston movement. The blood is pumped through a fine mesh filter (pore diameter: 89 μm) and enters the kidney via the arterial line. At the entrance of the artery, a polyethylene tube is connected, which transmits the hydrostatic

pressure to a Gould statham pressure transducer (P23 ID). Blood leaves the kidney at the venous side, first entering a blood flow sensor (Skalar Transflow 600 System, extracorporeal probe, ID 3 mm) and subsequently flowing on the rotating cylinder in the oxygenator.

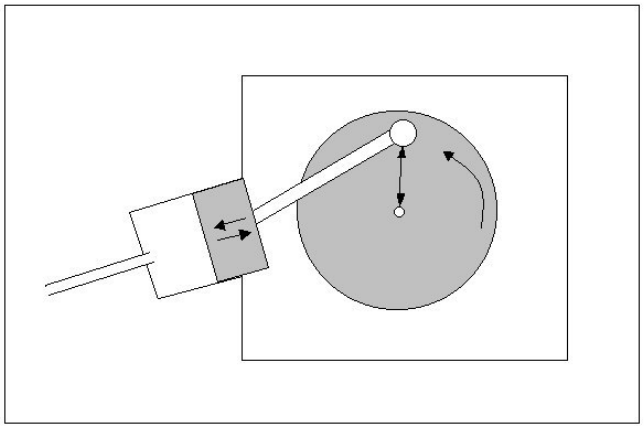

Figure 3.1: Scheme of pulse generating device.

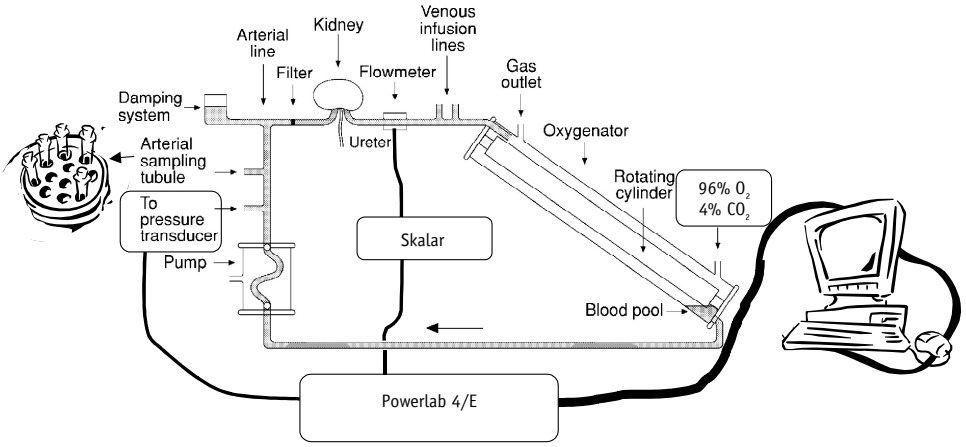

Figure 3.2: Perfusion setup. Adapted from Cuypers and co-workers (Cuypers et al., 2000).

The oxygenator consists of a plexyglass tube, with a concentric pvc cylinder (length: 30 cm, diameter 31 mm) rotating in it (20 – 30 rpm). The whole is mounted in a water bath under an angle of about 30°. Blood flows in at the upper end on the rotating cylinder and is distributed over the surface as a very thin film. At the lower end the blood is collected in

the tube and drained via an outlet to the pump. A gas mixture of 96% O_2 and 4% CO_2, saturated with water, enters the plexiglass tube at the lower end and leaves the closed tube at the upper end. Pump, filter and oxygenator are kept in a warm water bath (38 °C).All connections are made with silicone tubes, ID 3.0 mm, and OD 6.0 mm.

At the venous end a continuous infusion is given at a rate of 0.1 ml/min in order to compensate for losses of fluid, electrolytes and substrates in urine and due to metabolism. The infusion solution contained (mmol/l): NaCl 35, KCl 40, creatinine 7.4, urea 133, NaH_2PO_4 3.4, Na_2HPO_4 13.6, $NaHCO_3$ 22.6, glutamine 10, glucose 55, and [arg^8]-vasopressin, (AVP, Sigma) 30 IU/l. Osmolality was 422 mOsm/kg. The composition of this infusion solution has been based on the contents of the urine in pilot experiments. When urine flow exceeded the rate of infusion, an extra infusion of 0.45% - 0.9% NaCl solution was started, depending on the osmolality of the urine.

Blood flow was continually monitored and figure 3.5 is an example of the recording trace.

3.3 Course of the experiment

Perfusion pressure was constantly kept at 100 mmHg by varying blood flow. Blood flow can be varied by changing the amplitude of the piston movement. In this way only the stroke volume of the system changes, pulsating frequency remains constant at a rate of 163 bpm.

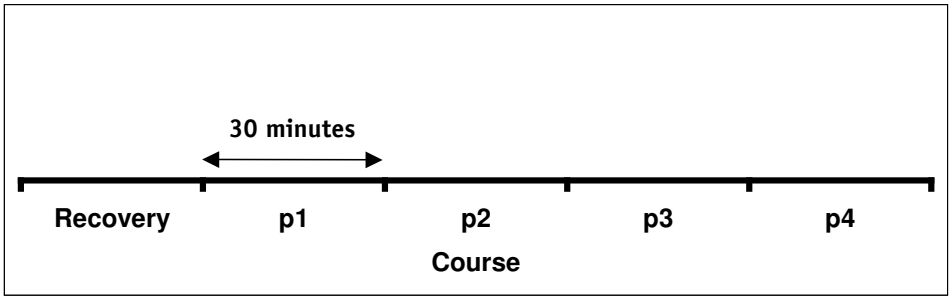

Figure 3.3: Regular course of experiments with four perfusion intervals.
Perfusion is started immediately after all connections have been made, 3 - 5 minutes after the kidney has been extirpated.
After perfusion was started, the kidney is allowed to recover from the stress it has undergone during the operation (figure 3.5).

During a period of 30 - 40 minutes urine production and renal blood flow are monitored. After this so called recovery period, urine and blood collections are started.
Urine is collected in four periods of 30 minutes each. Blood (2 ml) is collected at the beginning of each urine-collecting period and after the last period (figure 3.3). Identical volumes of blood from the same animal, which had been exsanguinated in the meantime, replaced all blood samples. Blood losses by bleeding (rare) were also replaced. Blood replacement was done very carefully, preventing reactive vasoconstriction.

3.4 Analyses

Plasma and urine samples were analysed for Na^+, K^+, Cl^-, Ca, urea and creatinine by a Beckmann Synchron Clinical System CX4. Glucose was determined by a glucose oxidase method (Biotrol Diagnostic). Plasma haemoglobin was measured with an Abbot Cell-Dyn 3500. pH, PCO_2 and PO_2 were measured with a Chiron Diagnostics 238 pH/Blood Gas Analyser (Bayer). Osmolality was measured using a Knauer Halbmikroosmometer. The haematocrit was determined after centrifugation (Cuypers et al., 2000).
Immediately following the perfusion the kidneys were decapsulated and weighed after vessels and ureter had been removed as far as possible.

3.5 Intratubular pressure measurements through micropuncture

In three series of experiments, pressure in the proximal tubules was measured according to the technique of Wiederhielm and Fein (figure 3.4) (Wiederhielm et al., 1964; Fein, 1972). For this purpose, a servo nulling micropressure system (Model 900A, WPI, Boca Raton, Fla., USA) was used. This system is based on the following principle. A measuring electrode is filled with a low resistance solution, for example 3M KCl. The electrode tip (2 – 5 μm) then has a typical electrical resistance, which is determined in a 0.9% NaCl solution.
When entering the electrode in a structure as the proximal tubule, the hydrostatic pressure of the fluid forces intratubular fluid into the tip of the electrode. This will increase the electrical resistance of the electrode, which is signalled by the device. In order to re-establish the electrode resistance, the device will create a counter pressure that is equal to the hydrostatic pressure in the proximal tubule. This pressure is then displayed as the intratubular pressure and recorded digitally with a Powerlab 4/E system.
Tubules were visualised with microscopic aid (Leica stereo microscope 10 X 6 – 10 X 40) and were identified on morphological aspects. In addition, potential difference was

concomitantly measured and aided when visibility was limited. In a certain number of experiments lissamine green (0.2 – 1.0 g/100 ml saline) was used to identify the proximal tubules, but after administration of the material a rapid decline in renal function was observed and the use of it was discontinued.

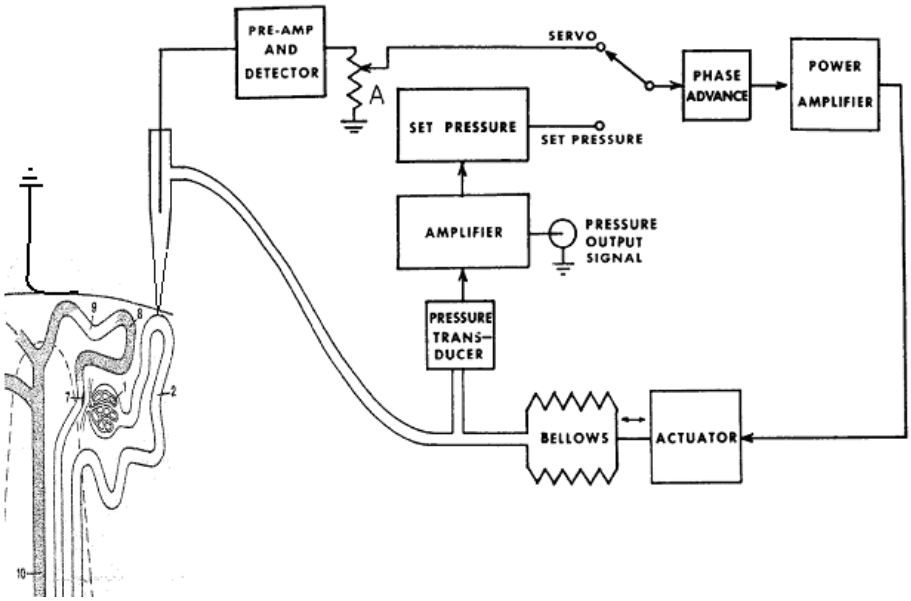

Figure 3.4: Schematic presentation of the principles of hydrostatic pressure measurements in the proximal tubule. Adapted from (Fein, 1972).

For obvious reasons, when hydrostatic pressure had to be measured, the kidney was superfused with warm saline instead of oil.

3.6 Statistics

Results are presented as estimated mean ± SE. Data are statistically analysed within the framework of the linear mixed model, in contrast to the usually applied fixed model. Complex data structures can be described in a natural way in the mixed model. The classical fixed effects model cannot handle random effects.

In addition the mixed model framework allows a flexible choice of the appropriate inference space. When wrongly using the fixed effects model to analyse data in which more than one source of random variations occurs, the calculated standard errors of the means do not take into account the different sources of variation. Within the framework of the mixed model,

the appropriate standard errors can be derived, since the different sources of variation can be included in the derivation of the standard error (Duchateau et al., 1998; Verbeke and Molenberghs, 2001).

The SAS system for Window® V8 (© 1999 – 2001 by the SAS institute Inc., Cary, NC, USA) was used to analyse the aquired data.

3.7 Histology

In a few experiments, the kidney was fixed for histological examination at the end of the perfusion. The arterial circulation was interrupted and about 15 ml formalin (6%) was injected intra-arterially at a constant pressure of 100 mm Hg. The kidney was then cut into pieces and the pieces fixed further in formalin. Sections were stained with trichrome (Masson) and haematoxylin-eosin reagents.

3.8 Parameters in the conscious rabbit

In a group of conscious rabbits blood was sampled from an ear artery. Urine was collected through a puncture of the baldder, after anesthesia when the abdomen was opened. Blood and urine were analyzed as described above.

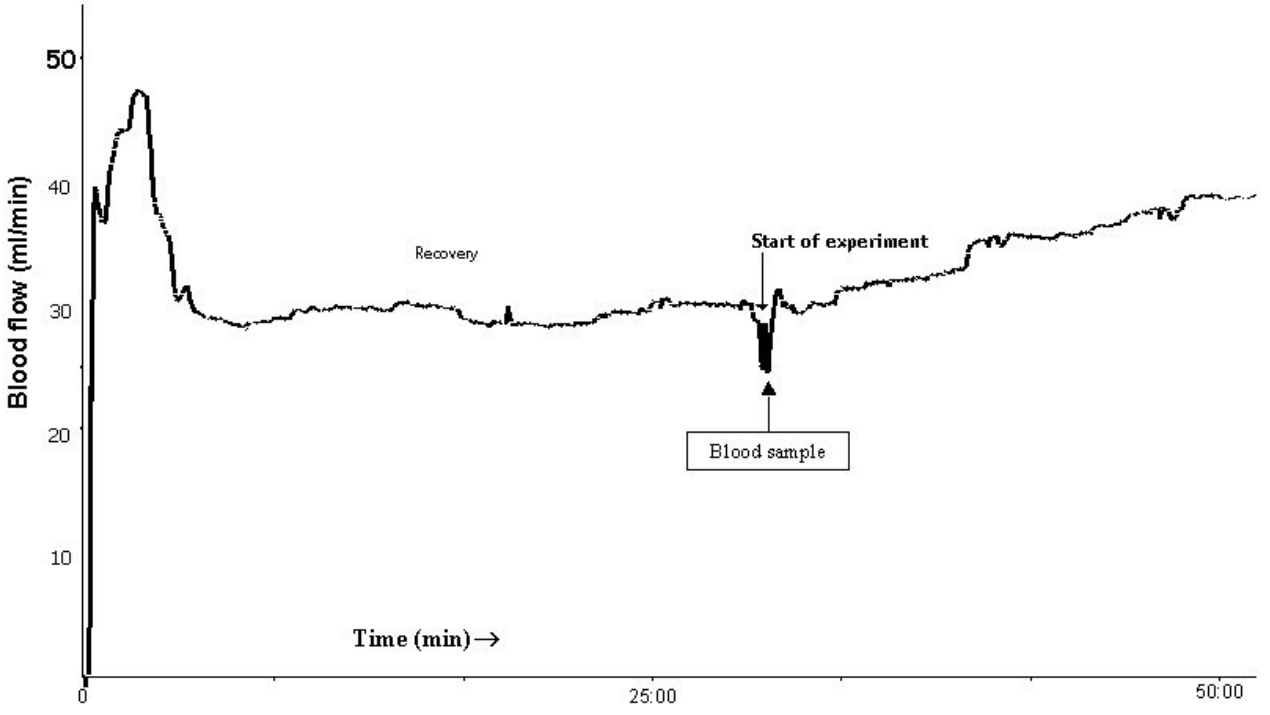

Figure 3.5: Typical recording of renal blood flow.

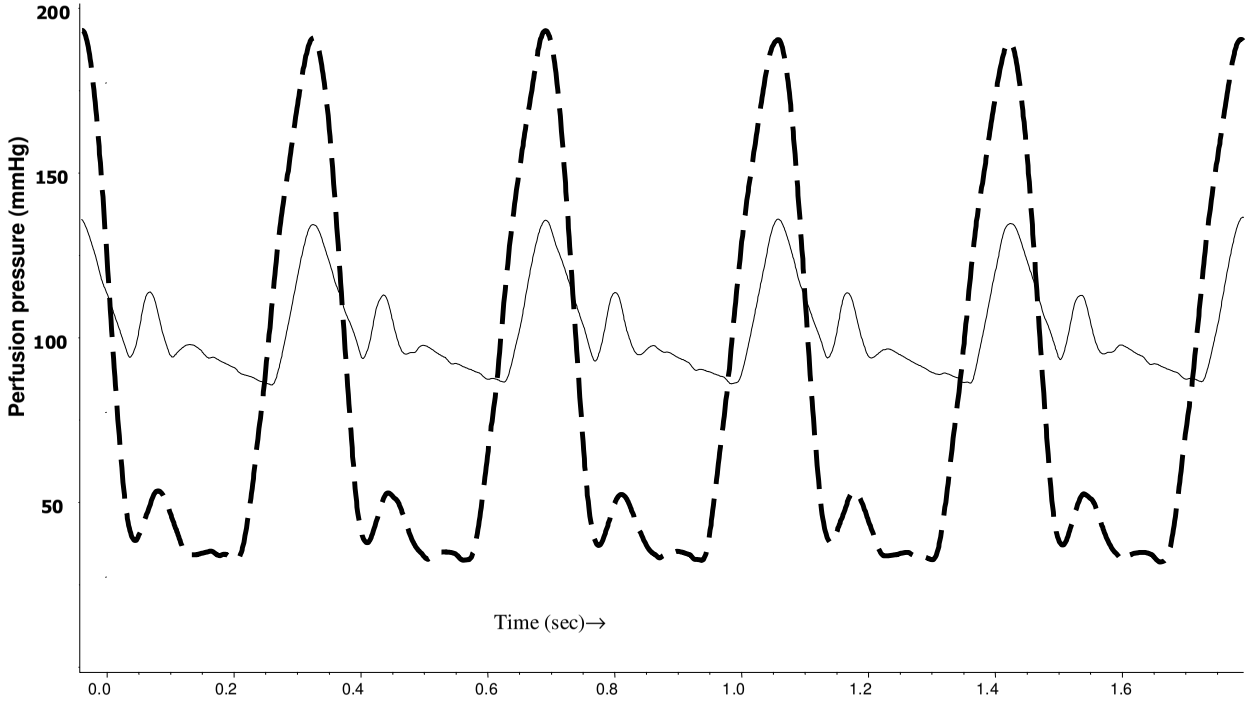

Figure 3.6: Perfusion pressure as recorded when damping is present (straight line) or absent (dashed line)

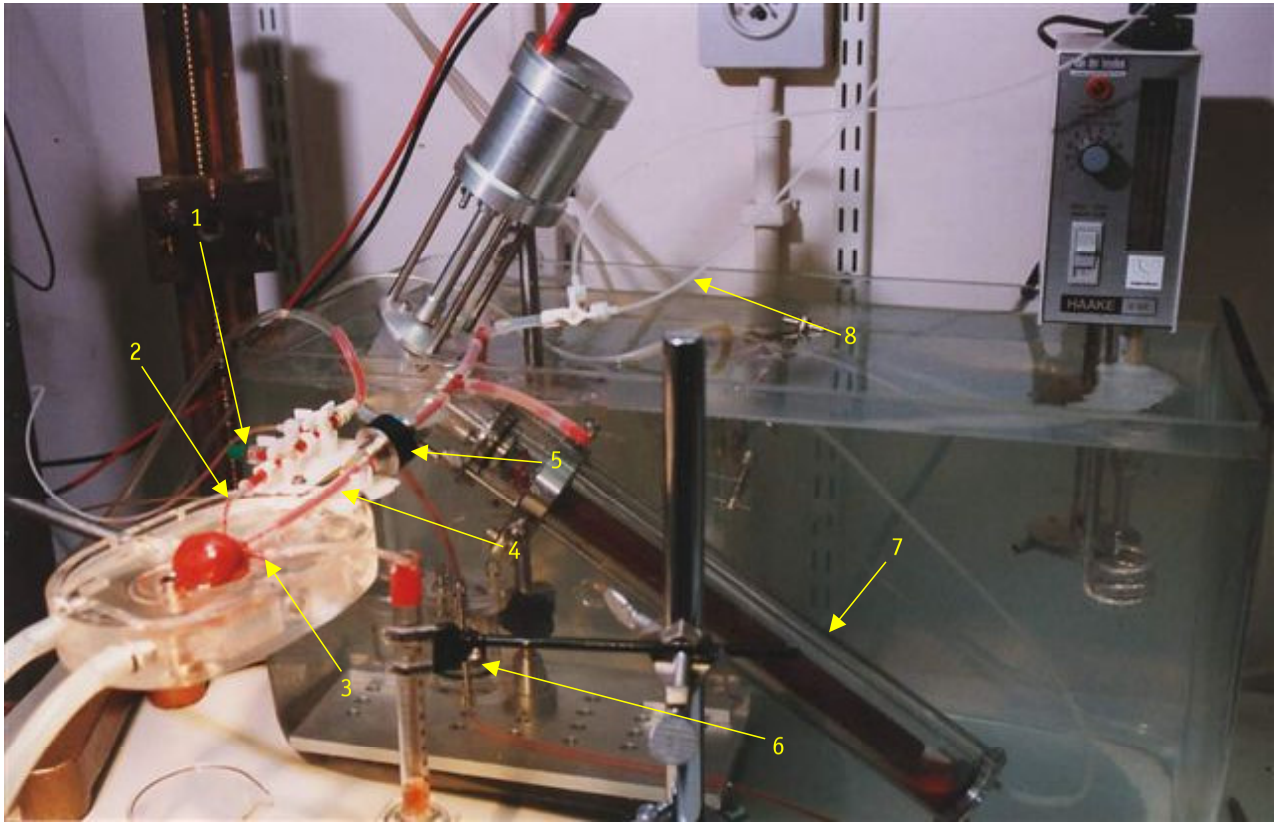

Figure 3.7: Colour photograph of the experimental setup. 1: arterial sampling line, 2: arterial line, 3: ureter, 4: venous line, 5: flow sensor, 6: pump, 7: oxygenator, 8: infusion line.

Chapter 4: Hemodynamic, tubular and concentrating properties of the isolated perfused rabbit kidney[1]

[1]Part of this chapter has been published in: Cuypers, Y., Vandenreyt, I., **Bipat, R.**, Toelsie, J., Van Damme, B., and Steels, P. The functional state of the isolated rabbit kidney perfused with autologous blood. *Pflügers Arch* 440: (634-642) 2000.

Hemodynamic, tubular and concentrating properties of the isolated perfused rabbit kidney

4.1 Introduction

A description of the normal function of the kidney focuses on renal blood flow, glomerular filtration rate, excretion and reabsorption of electrolytes and glucose as well as concentrating ability. Some parameters are not measured directly, but are calculated from measured values. An overview of the important calculated parameters follows.

Glomerular filtration rate (GFR) is assessed as the clearance of creatinine and is calculated as follows:

$CrClearance = \frac{V_u \times U_{cr}}{P_{cr}}$, in which Vu = urine volume/min.g kw, U_{cr} = urinary creatinine concentration and P_{cr} = plasma creatinine concentration.

An indirect way to estimate the **effect of aldosterone** would be to measure the tubular fluid $\mathbf{K^+}$ concentration at the end of the cortical collecting tubule, after most of the K^+ secretion has occurred. This can be estimated if the following assumptions are correct: the urine osmolality at this site is similar to that of the plasma, since equilibration with the isosmotic interstitium will occur in the presence of AVP and little or no K^+ secretion or reabsorption takes place in the medullary collecting duct. In this setting, the luminal K^+ concentration and U_{K^+} / P_{K^+} ratio will rise in the medulla because of the reabsorption of water. This can be accounted for by dividing the K^+ ratio by the ratio of urine and plasma osmolality. These assumptions yield the following equation:

$TTKG = \frac{(U_{K^+}) \times (P_{osm})}{(U_{osm}) \times (P_{K^+})}$, the **t**rans**t**ubular potassium ($\mathbf{K^+}$) **g**radient or ***TTKG***.

The estimation of this **dimensionless parameter** is relatively accurate as long as the urine is not dilute and the urine Na^+ concentration is above 25 mEq/l, so that Na^+ delivery is not the limiting factor. The TTKG in subjects with a regular intake of potassium is 8 – 9, whereas it rises to above 11 with an increased potassium load, indicating K^+ secretion. A value below 7 in combination with a hyperkalemia is highly suggestive for hypoaldosteronism (Rose, 1994).

Another parameter to consider with respect to **renal concentrating ability** is the free water clearance:

$$C_{H_2O} = V_u - C_{osm} \quad \text{in which} \quad C_{osm} = \frac{V_u \times U_{osm}}{P_{osm}}$$

(U_{osm}, urine osmolality; P_{osm}, plasma osmolality; V_u, urine volume/min). C_{H_2O} becomes negative and is denoted as $T^C{}_{H_2O}$, true water reabsorption, when the urine is concentrated. The value of $T^C{}_{H_2O}$ increases when C_{osm} increases, which indicates that the concentrating ability of the kidney rises when the excretion of osmolytes increases (Jamison and Kriz, 1982).

Lastly, **fractional sodium or potassium excretion** in conscious animals can be estimated from the following equation:

$$\frac{excretion}{load} = \frac{(U_{ion}) \times (V_u)}{(GFR) \times (P_{ion})} = \frac{(U_{ion}) \times (V_u)}{\left(\frac{(V_u) \times (U_{cr})}{(P_{cr})} \times (P_{ion}) \right)} = \frac{(U_{ion}) \times (P_{Cr})}{(U_{Cr}) \times (P_{ion})}$$

(U_{cr}, urine creatinine; P_{cr}, plasma creatinine; V_u, urine flow)

Using this formula bypasses the need for measuring urine volume.

4.2 Methods and protocols

In order to evaluate in vitro experiments, results are compared with the in vivo situation. Therefore, some data were obtained from conscious rabbits (arterial blood, bladder urine). General materials and methods have been presented in chapter 3. When a different method was applied, it will be explicitly mentioned.

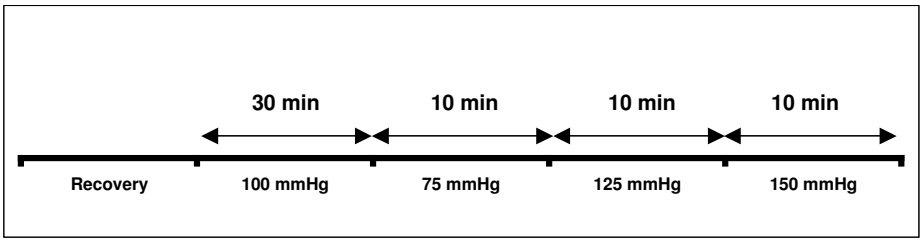

Figure 4.1: Protocol of the experiments performed with different perfusion pressures. The sequence was always the same.

In eight experiments the normal course of renal blood flow and GFR under control circumstances (100 mmHg) was established, as well as tubular handling of sodium, potassium and urea and the ability of the kidney to concentrate the urine.

In a series of five experiments renal perfusion pressure was varied in the second period of perfusion. This period was divided in three equal periods of ten minutes. In each ten-minute period the pressure was set to 75 mmHg, 125 mmHg and 150 mmHg, respectively (figure 4.1).

Due to the results in these experiments, another series of five experiments was performed in which the perfusion pressure was set to a constant value of 125 mmHg during the entire second period. Urine collection in this second period was divided over two periods of 15 minutes, thus GFR could be calculated over two periods of 15 minutes.

To investigate the concentrating ability of the kidney, six experiments were performed in which AVP had been withheld from the compensating infusion, while in another series of seven experiments urea had been omitted in the compensating infusion. When urine production exceeded the amount of fluid that was continuously being infused, an additional infusion of NaCl was started. The NaCl concentration of the extra infusion depended on the expected urine osmolality (0.45% - 0.9% NaCl).

Experiments were also performed using an infusion solution without phosphate and some experiments with extra phosphate (34 mmol/L).

In order to investigate renal transport properties of glucose in this preparation, the amount of glucose in the infusion solution was modified in two other series of experiments (0 - 85 mmol/L).

4.3 Results

4.3.1 General

Table 4.1: Arterial blood and plasma parameters in conscious rabbits. Blood was sampled from the ear artery.

	n=13		n=7-11		n=7-11
pH	7.31±0.03	**Na^+ (mM)**	143±1	**Urea (mM)**	6.7±0.2
P_{CO_2} (mmHg)	24.8±1.9	**K^+ (mM)**	3.6±0.3	**Glucose (mg/dl)**	115±7
P_{O_2} (mmHg)	81±2	**Ca^{2+} (mg/dl)**	13.6±0.1	**Creatinine (mg/dl)**	1.10±0.03
HCO_3^- (mM)	13.3±1.5	**Cl^- (mM)**	100±1	**Anion gap (mM)**	28.4±2.2
		P (mg/dl)	6.0±0.3	**Osmolality (mOsm/kg)**	301±7
				Haematocrit (%)	38.3±0.8

Values are mean ± SEM.

Table 4.1 presents arterial blood (obtained from the ear artery) and plasma parameters in conscious rabbits, while table 4.2 illustrates values in urine of the same rabbits.

Table 4.2: Composition of bladder urine as well as estimated TTKG and fractional excretion of sodium and potassium.

Na^+ (mM)	133±18	**Creatinine (mg/dl)**	78±17
K^+ (mM)	178±28	**Urea (mM)**	306±23
Ca^{2+} (mg/dl)	31±22	**Glucose (mg/dl)**	18±7
Cl^- (mM)	93±18	**Osmolality (mOsm/kg)**	831±81
P (mg/dl)	37±15	**Estimated TTKG**	17.9
Estimated FE K (%)	70	**Estimated FE Na (%)**	1.3

Values are mean ± SEM (n = 7 – 11).

All further data regard the function of isolated kidneys.

Table 4.3: Blood hematocrit during four successive 30-minute periods (P1–P4).

Period	P1	P2	P3	P4
Haematocrit(%)	32 ± 1	33 ± 2	32 ± 2	33 ± 2

Table 4.4: Arterial blood and plasma data (n=8) in four, successive, 30-min urine collection periods.

Period	P1	P2	P3	P4
Ca (mg/dl)	9.4±0.3	9.0±0.4	8.6*±0.5	8.6*±0.5
Cl^- (mM)	97±2	93*±2	90*±2	88*±2
Creatinine (mg/dl)	2.53±0.24	2.49±0.18	2.53±0.18	2.86±0.26
pH	7.37±0.02	7.34±0.02	7.33*±0.03	7.34*±0.02
P_{CO_2} (mmHg)	42±1	42±1	41±1	42±1
P_{O_2} (mmHg)	123±13	118±13	137±15	122±8
HCO_3^- (mM)	24±1	22±1	21±1	22±1
Anion gap (mM)	17±1	18±1	18±1	19±1

Plasma Na^+ is presented in table 4.5 (control). Values are the mean of two successive blood collections ± SEM, *P<0.05 vs. P1.

Measured parameters that will not be discussed in the text, but are valuable information to indicate proper function of the kidney are presented in table 4.4.

The courses of the renal blood flow (RBF) and glomerular filtration rate (GFR, creatinine clearance) under standard (100 mmHg) control condition have been graphically plotted in figure 4.2. RBF rises almost linearly throughout the experiments, from 2.0 ± 0.2 ml/min.g kw to 2.7 ± 0.3 ml/min.g kw. Despite this rise in RBF, GFR remains stable in the first two periods (288 ± 27 and 289 ± 44 µl/min.g kw) and then declines, becoming only significantly lower in the last period (217 ± 33l/min.g kw). These trends are observed constantly over the whole range of performed experiments.

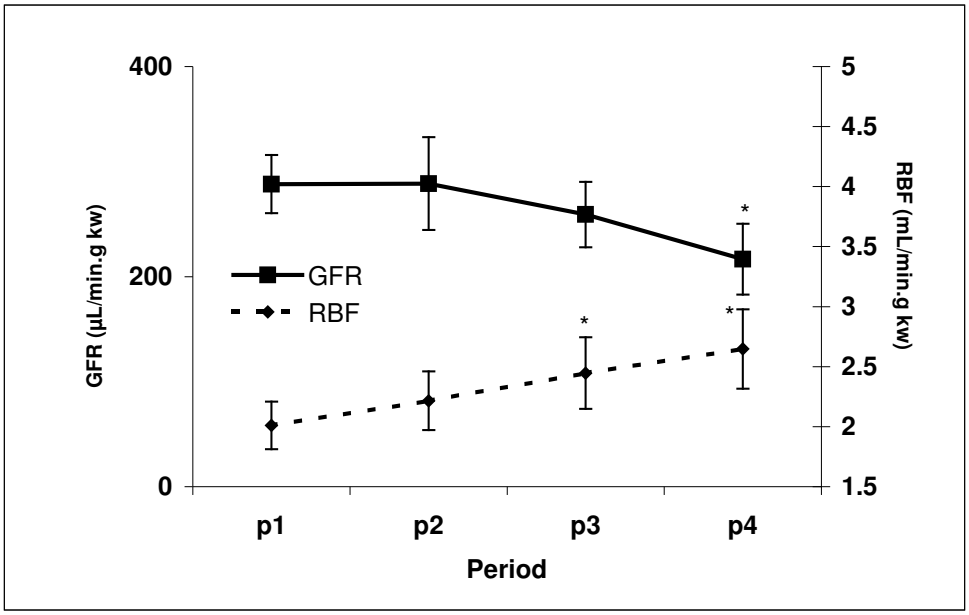

Figure 4.2: Renal blood flow and GFR under regular perfusion conditions. Perfusion pressure is constantly kept at 100 mmHg. * p<0.05 vs. P1.

4.3.2 Effect of perfusion pressure on hemodynamics

Figures 4.3 and 4.4 compare the renal blood flow between different perfusion pressures, while figures 4.5 and 4.6 do the same for GFR.

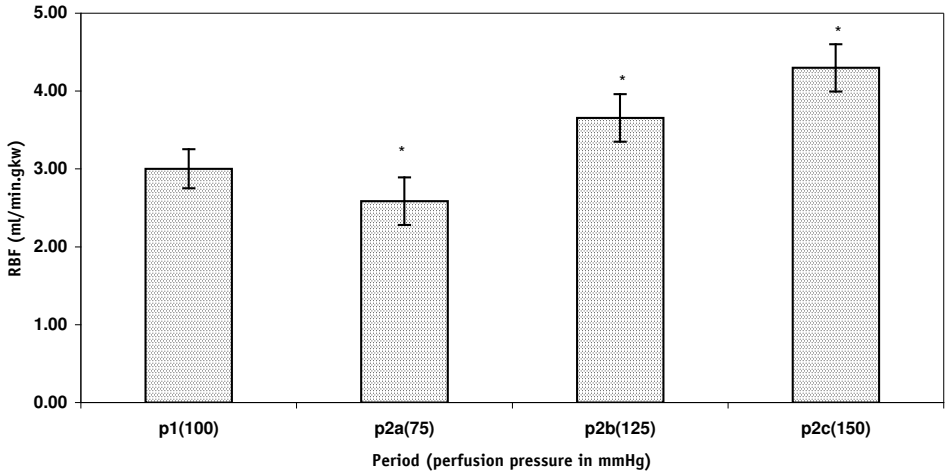

Figure 4.3: Renal blood flow with different perfusion pressures during P2. * p<0.05 vs P1.

Decreasing the perfusion pressure to 75 mmHg led to a simultaneous decrease of blood flow from 3.0 ± 0.25 ml/min.g kw to 2.6 ± 0.31 ml/min.g kw. Subsequent increase of perfusion pressure to 125 mmHg led to a rise of RBF to 3.6 ± 0.31 ml/min.g kw. Further increasing perfusion pressure to 150 mmHg resulted in RBF of 4.3 ± 0.31 ml/min.g kw (figure 4.3). All these values were significantly different from the control initial value in P1 of 3.0 ± 0.25 ml/min.g kw.

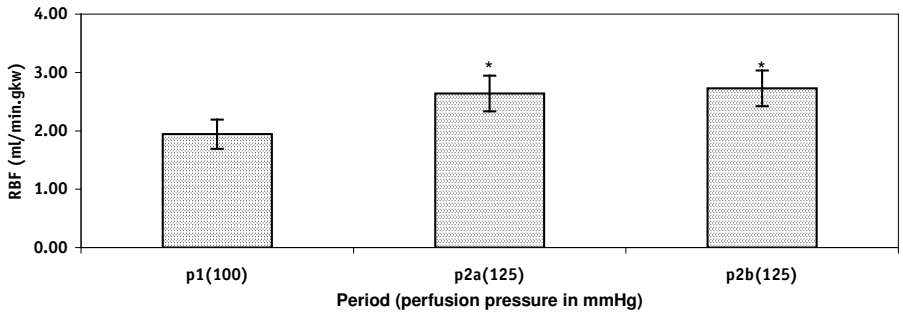

Figure 4.4 : Renal blood flow with a constant increased pressure of 125 mmHg during P2. *p<0.05 vs P1.

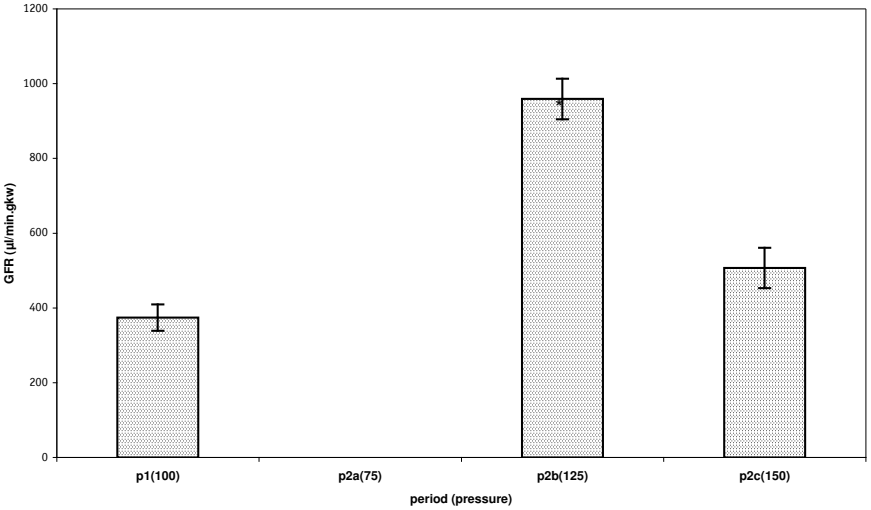

Figure 4.5: GFR with different perfusion pressures. * p<0.05 vs P1, $ p<0.05 vs control series. When kidneys were perfused at 75 mmHg, almost no urine production in ten minutes was collected, making it impossible to estimate GFR.

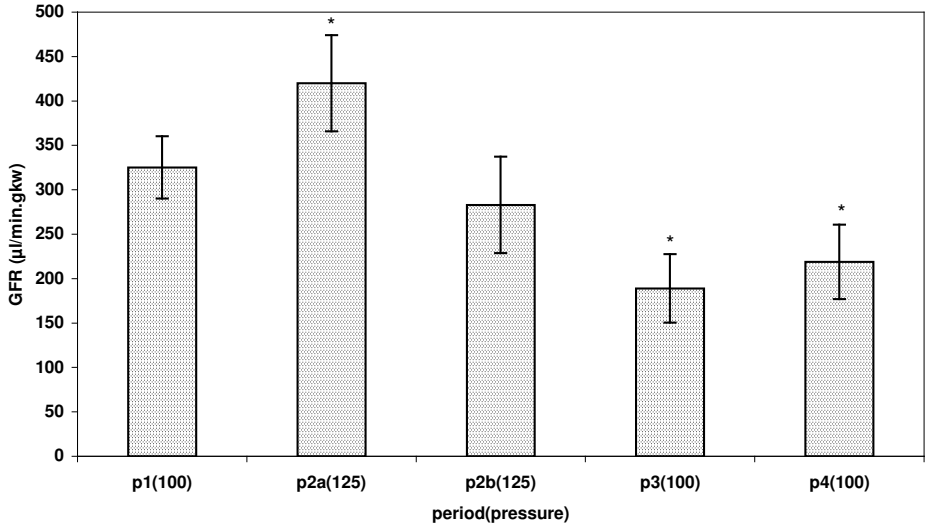

Figure 4.6: GFR with a constant increased perfusion pressure of 125 mmHg during P2. * p<0.05 vs P1.

Glomerular filtration rate rose from a value of 341 ± 35 µl/min.g kw in the first period to 679 ± 54 µl/min.g kw with an increase of perfusion pressure to 125 mmHg. With a further rise of the perfusion pressure to 150 mmHg this GFR declined to 402 ± 54 µl/min.g kw (figure 4.5). The latter was not significantly different from the control value in P1. After returning to normal (100 mmHg) perfusion pressure, GFR became very low. In fact there was so little urine production in the third period that it was combined with P4 to make a reasonable estimation (157 ± 41 µl/min.g kw, not shown in the graph), meaning that the GFR was estimated over a period of 60 minutes. During the perfusion periods in which the pressure was set at 125 and 150 mmHg there was enough urine production so that dead space did not matter in these conditions and GFR could be safely estimated.

Because of the previous results of the GFR, (damaging influence of the high perfusion pressure?) it was decided to perform another series of experiments in order to evaluate the influence of a constant high perfusion pressure (125 mmHg) during P2.

RBF remained significantly higher during P2 (2.6±0.3 ml/min.g kw and 2.7±0.3 ml/min.g kw) (figure 4.4) and returned to control values during P3 and P4 (2.3 ± 0.4 ml/min.g kw and 2.6 ± 0.4 ml/min.g kw respectively, not shown in the graph).

The GFR increased significantly from 325 ± 35 µl/min.g kw to 420 ± 54 µl/min.g kw in the first half of P2 (15 minutes) after the perfusion pressure had been increased to 125 mmHg, but declined in the second half of P2 (minute 16 – 30) to a value close to that of P1 (283 ± 54 µl/min.g kw). When perfusion pressure was set again to 100 mmHg (P3), GFR was significantly lower (189 ± 39 µl/min.g kw) than control, but increased again during P4 (219 ± 41 µl/min.g kw) and did not differ any more from the lower value in the control series (figure 4.6). Contrary to the previous series, there was enough urine flow in both P3 and P4.

4.3.3 Effect of AVP and urea on RBF and GFR

Renal blood flow in the absence of AVP rose faster than in the presence of AVP during the normal course of experiments. RBF in P2 (2.6 ± 0.3 ml/min.g kw) was already significantly different from P1 (2.0 ± 0.2 ml/min.g kw) when AVP was left out of the compensating infusion (figure 4.7), suggesting that AVP might induce vasoconstriction. GFR was not affected and followed the same pattern as in the control series, not differing significantly in the absence of AVP (figure 4.8).

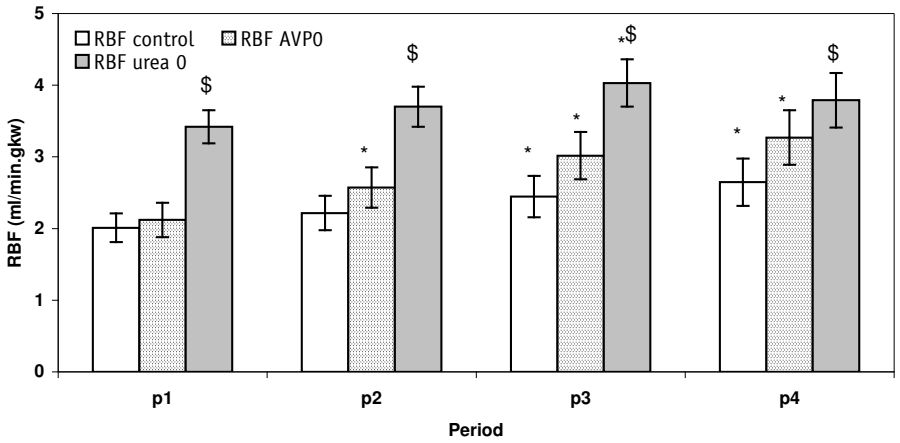

Figure 4.7: Renal blood flow under three different conditions: Control experiments were performed in the presence of AVP and urea in the compensating infusion; AVP0: AVP absent, urea present; urea 0: AVP present, urea absent. * p<0.05 vs. P1, $ p<0.05 vs. control series.

In the absence of urea, but presence of AVP, renal blood flow increased gradually from 3.4 ± 0.2 ml/min.g kw to 4.0 ± 0.3 ml/min.g kw in P3. In the last period an insignificant decrease to 3.8 ± 0.4 ml/min.g kw was observed (figure 4.7). On the other hand, GFR followed about the same pattern as in the other two conditions i.e. a gradual decrease in P3 and P4 (figure 4.8).

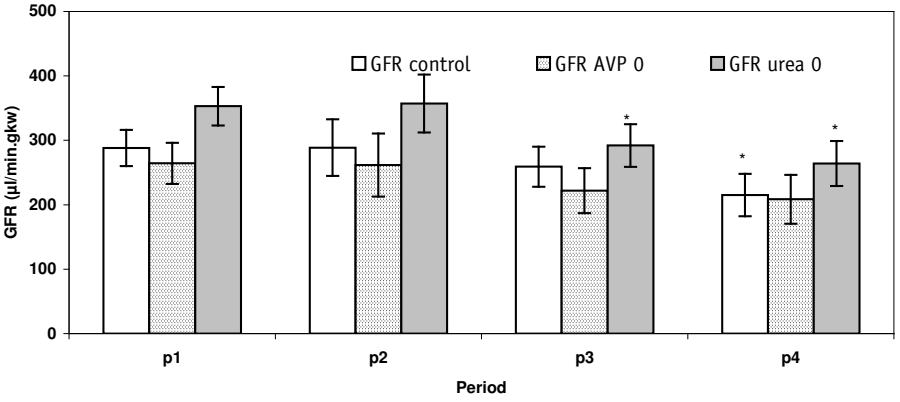

Figure 4.8: GFR in the presence (control) and absence of arginine vasopressin (AVP0) and urea (urea 0). * p<0.05 vs. P1.

As can be deduced from figure 4.2, that shows a decline in GFR and a linear rise in RBF, it can be predicted that the filtration fraction ($\frac{GFR}{RPF}$), ($RPF = RBF \times (1 - Hct)$, Hct = Hematocrit), will decrease towards the end. This is demonstrated in figure 4.9, where there is a rather constant filtration fraction in P1 and P2 (21 ± 1%, 20 ± 2%, respectively), but with a decrease that reaches 13 ± 3% in P4.

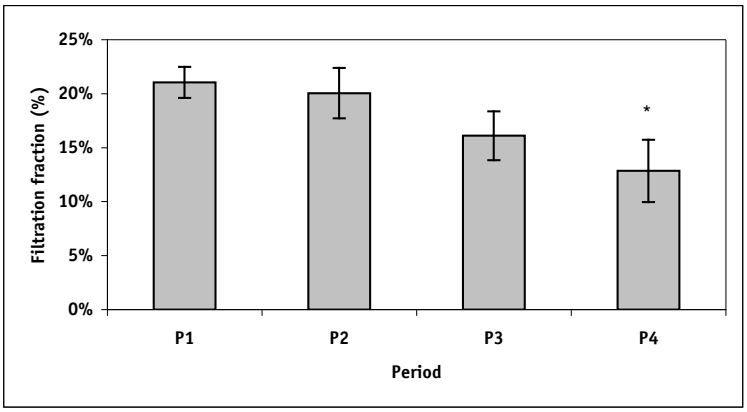

Figure 4.9: Filtration fraction in four periods under regular perfusing conditions. Values are mean ± SEM. *p<0.05 vs. P1.

4.3.4 Effect of perfusion pressure on water and sodium excretion

Diuresis and sodium excretion under different perfusion pressures are presented in figure 4.10. With a perfusion pressure of 75 mmHg, no urine flow could be observed during the ten minutes of observation, hence there are no data. An almost perfect linear relationship can be observed between perfusion pressure and water or sodium excretion.

4.3.5 Effect of AVP and urea on water and sodium excretion

In table 4.5 handling of sodium is presented under regular perfusion conditions and in the absence of arginine vasopressin or urea. Excretion of water under the three different perfusion conditions is presented in figure 4.11, while fractional excretion of sodium is presented in figure 4.12.

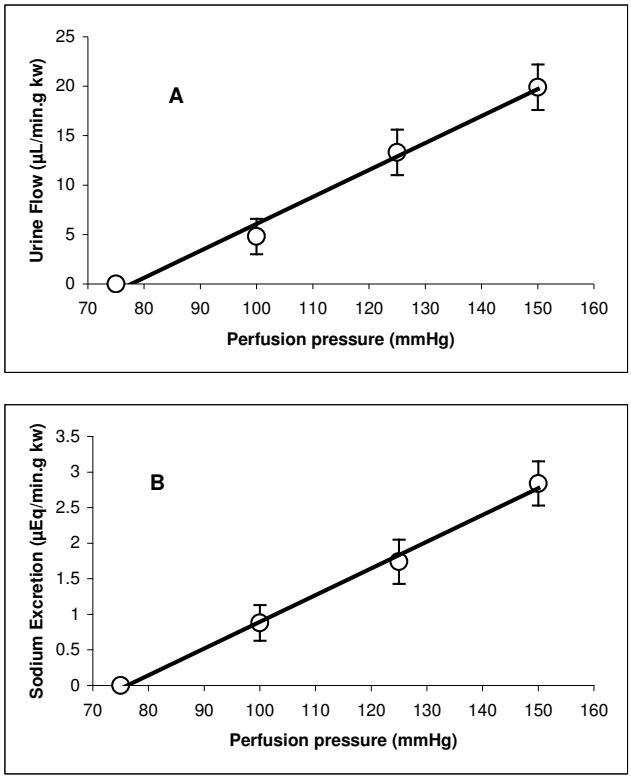

Figure 4.10: Effect of perfusion pressure on water (A) and sodium (B) excretion. Plots represent estimated mean ± SE in a series of 5 experiments.

Table 4.5: Excretion parameters of sodium.

	Period	P1	P2	P3	P4
Control (n = 8)	Plasma Na^+ (mmol/l)	137±1	133±2*	131±2*	129±2*
	Filtered Na^+ (µEq/min)	39±4	38±4	34±4	28±4*
	Na^+ excretion (µEq/min)	1.07±0.31	0.88±0.32	0.64±0.32	0.62±0.32
AVP 0 (n = 6)	Plasma Na^+ (mmol/l)	143±2$	143±2$	140±2$	138±3*$
	Filtered Na^+ (µEq/min)	38±4	38±5	31±5	29±5
	Na^+ excretion (µEq/min)	1.86±0.36$	1.97±0.36$	1.85±0.36$	2.01±0.36$
Urea 0 (n= 7)	Plasma Na^+ (mmol/l)	135±2	129±2*	126±2*	124±2*
	Filtered Na^+ (µEq/min)	47±4	46±4	37±4*	33±4*
	Na^+ excretion (µEq/min)	1.94±0.33$	1.54±0.33	1.27±0.33	1.39±0.33

***p < 0.05 vs. P1, $p < 0.05 vs. control.**

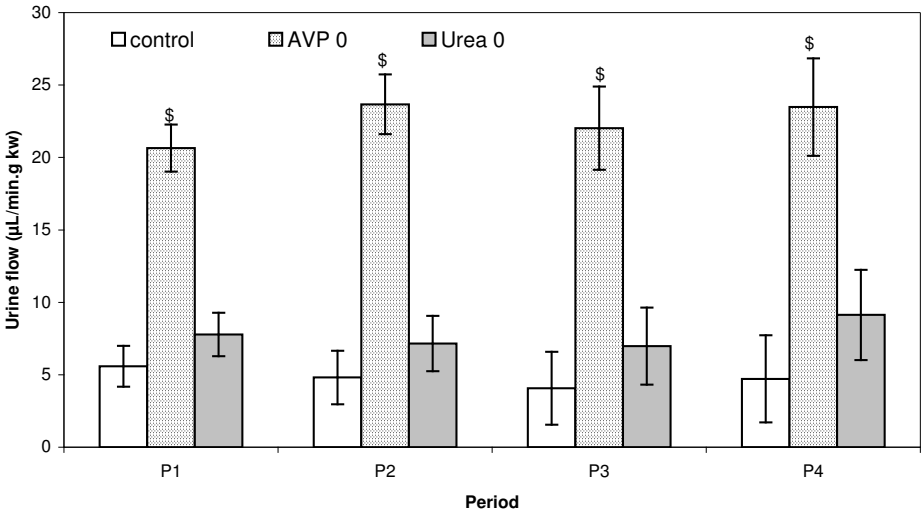

Figure 4.11: Urine flow under regular perfusion conditions (control) and in the absence of arginine vasopresssin (AVP 0) or urea (urea 0). Values are means ± SE, $ p<0.05 vs. control series.

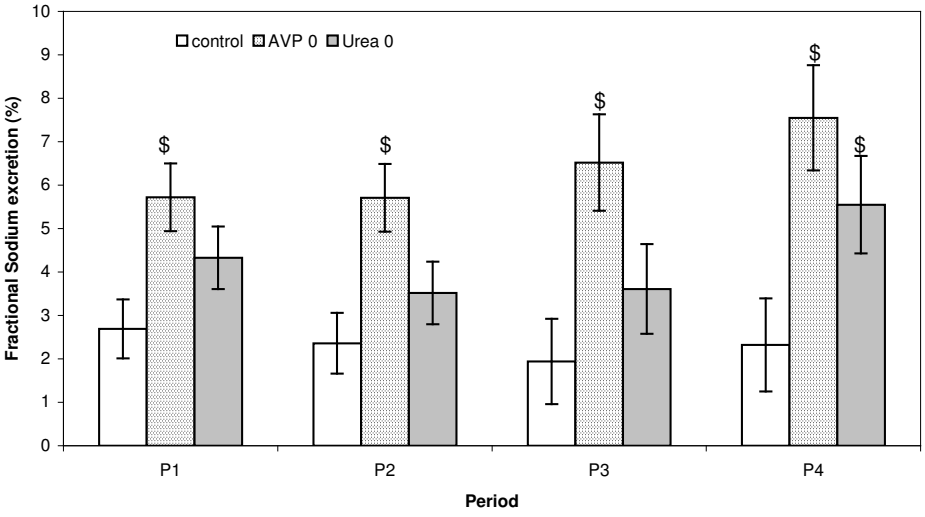

Figure 4.12: Fractional excretion of sodium under regular perfusion conditions and in the absence of arginine vasopressin or urea. Values are mean ± SE, $ p<0.05.

All three experiments show a decrease of plasma sodium towards the end, which is more pronounced in the presence of AVP, with or without urea. Filtered sodium does not differ between the groups.

Sodium excretion in the presence of AVP and urea is significantly less than in the absence of AVP. In the absence of urea, this difference is only significant during P1, but has a tendency to remain higher than in the control group. As a consequence, fractional excretion of sodium in the experiments performed without AVP in the infusion liquid is significantly higher than under regular perfusion conditions. In the absence of urea the increase in FE of Na^+ is not significantly different from the control series, except in P4.

4.3.6 Phosphate

Table 6 presents phosphate handling under regular perfusion conditions by this preparation. The plasma value of phosphate is slightly higher than the in vivo value (table 4.1) and increases slightly throughout the experiments.

Table 4.6: Phosphate handling in eight experiments.

Period	P1	P2	P3	P4
Plasma [P_i] (mg/dl)	6.4 ± 0.4	6.7 ± 0.4	7.2 ± 0.5	8.1 ± 0.7*
Filtered P_i (µg/gkw.min)	18.4 ± 1.1	19.9 ± 1.8	19.4 ± 2.0	18.0 ± 2.6
P_i Excretion (µg/gkw.min)	2.5 ± 0.2	2.9 ± 0.2	2.6 ± 0.2	2.0 ± 0.5
Fractional excretion (%)	13.3±0.9	14.7±1.0	13.1±1.2	10.3±1.4*

***p < 0.05 vs. P1.**

Filtered phosphate and excretion remain relatively constant. Phosphate excretion is plotted against filtered phosphate in figure 4.13.

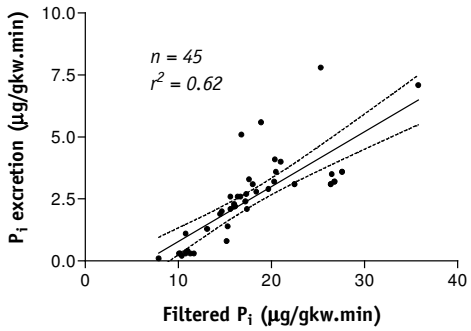

Figure 4.13: Phosphate excretion plotted against filtered P_i. Plotted points are the result of experiments performed with normal phosphate infusion, and with low and extra high infusion.

There is a slight linear relation between phosphate excretion and the filtered value. Moreover, one can also see that when filtration is low, almost all phosphate is reabsorbed. From a certain load on excretion starts, suggesting a transport maximum for reabsorption. However a single maximum of transport could not be determined.

4.3.7 Glucose

Glucose excretion under normal control conditions is presented in table 4.7. Remark that plasma glucose starts with an unphysiologically high value compared to the in vivo value (table 4.1), but declines to a more physiological value towards the end of the experiments. Glucose excretion reveals the same pattern. Glucose excretion is plotted against filtered glucose in figure 4.14. It appears that glucose excretion occurs only when plasma glucose largely exceeds the physiological ranges i.e. > 180 mg/dL.

Table 4.7: Glucose handling in eight experiments.

Period	P1	P2	P3	P4
Plasma glucose (mg/dl)	216 ± 16	194 ± 16	165 ± 18*	133 ± 21*
Filtered glucose (µg/gkw.min)	606 ± 47	548 ± 52	414 ± 52*	284 ± 67*
Glucose excretion (µg/gkw.min)	18.2 ± 3.2	9.4 ± 2.5*	4.3 ± 1.6*	1.6 ± 0.5*
Fractional Excretion (%)	3.1±0.8	1.7±0.4*	0.9±0.3*	0.6±0.1*

***p < 0.05 vs. P1.**

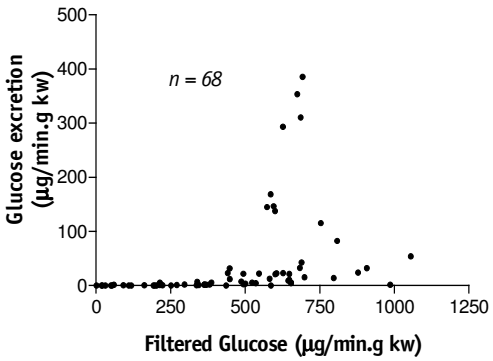

Figure 4.14: Glucose excretion plotted against filtered glucose. Data have been obtained through experiments performed with normal glucose or with an extra high or low infusion of glucose.

4.3.8 Effect of AVP and urea on potassium handling

Potassium excretion remains constant throughout the experimental periods (table 4.8). Plasma potassium starts low, but rises to a more normal value in the absence of urea. Potassium excretion decreases gradually in all three experimental settings.

Table 4.8: Potassium excretion parameters.

	Period	P1	P2	P3	P4
Control (n=8)	Plasma K^+ (mmol/l)	3.1±0.2	3.0±0.2	3.0±0.2	3.2±0.3
	Filtered K^+ (µEq/min)	0.84±0.09	0.86±0.09	0.78±0.08	0.69±0.09
	K^+ excretion (µEq/min)	0.46±0.05	0.38±0.05	0.32±0.03*	0.29±0.03*
AVP0 (n=6)	Plasma K^+ (mmol/l)	3.6±0.2	3.4±0.2	3.4±0.2	3.5±0.3
	Filtered K^+ (µEq/min)	0.95±0.10	0.89±0.10	0.73±0.09*	0.73±0.10*
	K^+ excretion (µEq/min)	0.39±0.06	0.38±0.06	0.32±0.03	0.32±0.04
Urea0 (n=7)	Plasma K^+ (mmol/l)	2.7±0.2	2.7±0.2	2.8±0.2	3.4±0.3*
	Filtered K^+ (µEq/min)	0.94±0.10	0.96±0.09	0.81±0.08	0.80±0.09
	K^+ excretion (µEq/min)	0.43±0.06	0.43±0.06	0.35±0.03	0.34±0.04

***p<0.05 vs. P1.**

4.3.9 Effectof AVP and urea on concentrating capacity

Under regular perfusion conditions, plasma urea starts rather high and rises towards the end of the experiments (table 4.1: 6.7 mM in plasma of conscious rabbits).

Table 4.9: Parameters of urea excretion.

	Period	P1	P2	P3	P4
Control (n=8)	Plasma Urea (mmol/l)	15.3±0.9	16.3±0.8	17.7±0.9*	18.1±1.3*
	Filtered Urea (µmol/min)	4.2±0.3	4.6±0.3	4.4±0.3	3.6±0.2
	Urea excretion (µmol/min)	0.76±0.12	0.84±0.15	0.89±0.08	0.95±0.10
AVP0 (n=6)	Plasma Urea (mmol/l)	10.2±1.0$	9.3±0.9$	8.7±1.1$	9.3±1.5$
	Filtered Urea (µmol/min)	2.4±0.3$	2.3±0.3$	1.9±0.3$	1.8±0.3$
	Urea excretion (µmol/min)	1.40±0.14$	1.30±0.17$	1.10±0.09*	1.10±0.11
Urea0 (n=7)	Plasma Urea (mmol/l)	3.2±0.9$	2.0±0.9$	1.6±1.0$	1.4±1.3$
	Filtered Urea (µmol/min)	1.1±0.3$	0.7±0.3$	0.4±0.3$	0.3±0.2$
	Urea excretion (µmol/min)	0.43±0.13	0.27±0.16$	0.16±0.08$	0.16±0.10$

***p<0.05 vs. P1, $p<0.05 vs. control series.**

On the contrary, this value remains within the normal range in the absence of AVP and as expected becomes very low in the absence of urea in the compensating infusion fluid. In

spite of the lower plasma concentration, fractional urea excretion is higher with omission of AVP or urea. This is illustrated in figure 4.15.

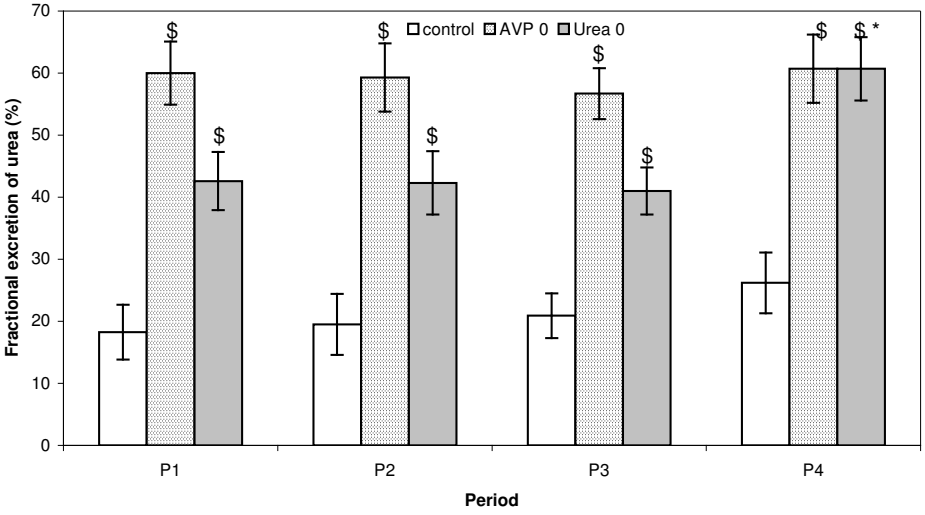

Figure 4.15: Fractional excretion of urea in different situations. AVP 0, absence of arginine vasopressin, Urea 0, absence of urea. Values are mean ± SE, *p<0.05 vs. P1, $p<0.05 vs. control series.

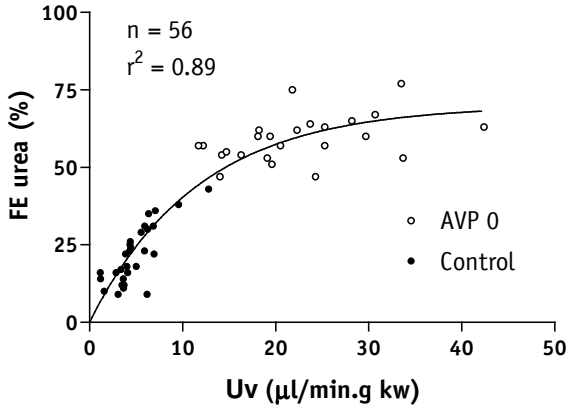

Figure 4.16: Relationship between urine flow (U_v) and fractional excretion of urea in the presence and absence of arginine vasopressin.

In figure 4.16 fractional excretion of urea is plotted against urinary flow. In the lower range of urine flow values (presence of AVP) there seems to be a proportional increase of

fractional excretion, but in the higher ranges (absence of AVP) this relation is lost and tends to saturate.

Figure 4.17 illustrates the urine osmolality in four consecutive periods. Clearly the kidney is able to concentrate and dilute, which is accentuated in figure 4.18 where the evolution of C_{H_2O} is shown in the four periods. Urine osmolality with regular perfusion remains constant, with a slight decrease towards the end of the experiments, which is not statistically significant.

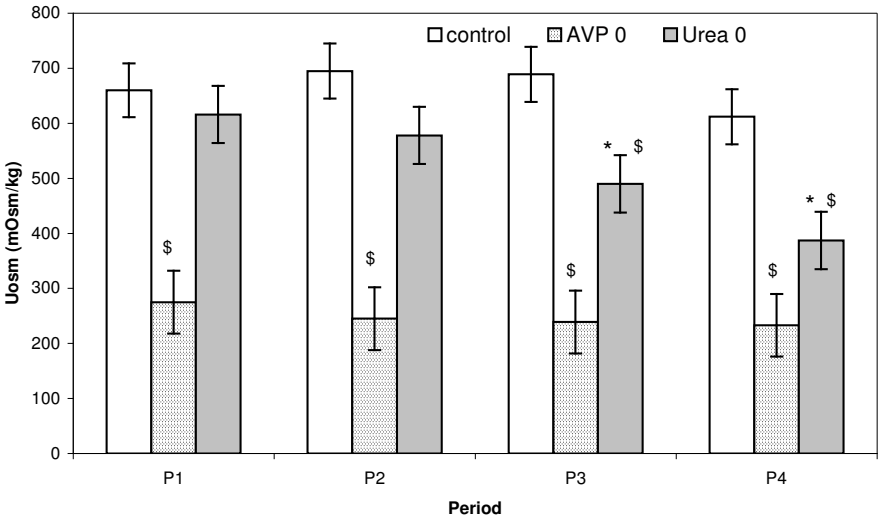

Figure 4.17: Urine osmolality in the four perfusion periods. Values are estimated means ± SE. *p<0.05 vs. P1, $p<0.05 vs. control series.

Table 4.10 illustrates plasma and urine osmolality as well as the relative contributions of sodium, potassium and urea to urine and plasma osmolality under regular perfusion conditions and in the absence of AVP or urea. Plasma osmolality measured with no urea in the infusion fluid starts low and declines fast, in contrast to the other two conditions starting at a higher value evolving with a gradual decrease. The contribution of sodium to plasma osmolality is more or less the same at the start of the three experiments, but becomes higher in the absence of urea. Relative contribution of sodium to urine osmolality increases in the absence of AVP and urea. The relative contribution of potassium to plasma

osmolality is the same in all the situations. However, its contribution to urine osmolality is substantially lower in the absence of AVP.

Table 4.10: Relative contribution of sodium, potassium, and urea to plasma and urine osmolality in four consecutive periods of urine collection.

	Period:	P1	P2	P3	P4
	Plasma osm (mOsm/kg)	285±4	279±5	274±5*	269±5*
	Plasma Na^+ (%)	48±0.5	48±0.6	48±0.8	49±0.5
	Plasma K^+ (%)	1.1±0.1	1.1±0.1	1.1±0.1	1.3±0.1
Control	**Plasma Urea (%)**	5.3±0.3	5.8±0.5	6.3±0.5	6.6±0.4
(n = 8)	**Urine osm (mOSm/kg)**	660±49	695±50	689±50	612±50
	Urine Na^+ (%)	30±2	26±3	22±3	23±3
	Urine K^+ (%)	15.0±2.1	13.8±1.9	13.5±1.2	10.9±0.7
	Urine urea (%)	21±2	29±2	37±3	36±2
	Plasma osm (mOsm/kg)	299±4$	293±6	289±6*$	284±5*
	Plasma Na^+ (%)	48±0.4	49±0.7	49±0.7	49±0.6
	Plasma K^+ (%)	1.2±0.0	1.2±0.1	1.2±0.1	1.2±0.1
AVP 0	**Plasma Urea (%)**	3.4±0.3	3.2±0.4	3.0±0.4	3.3±0.4
(n = 6)	**Urine osm (mOSm/kg)**	275±57$	245±57$	239±57$	233±57$
	Urine Na^+ (%)	33±1	34±1	36±1	37±2
	Urine K^+ (%)	6.9±0.6	6.9±0.5	6.5±0.7	6.4±0.7
	Urine urea (%)	26±3	26±3	22±1	24±2
	Plasma osm (mOsm/kg)	275±4	259±5*$	248±5*$	242±5*$
	Plasma Na^+ (%)	49.0±0.2	50.0±0.3	51.0±0.5	51.0±0.6
	Plasma K^+ (%)	1.0±0.1	1.0±0.1	1.1±0.1	1.4±0.2
Urea 0	**Plasma Urea (%)**	1.1±0.1	0.7±0.1	0.6±0.2	0.6±0.1
(n = 7)	**Urine osm (mOSm/kg)**	616±52	578±52	490±52*$	387±52*$
	Urine Na^+ (%)	43±3	39±1	39±1	41±1
	Urine K^+ (%)	10±1	11±1	11±1	10±1
	Urine urea (%)	10±1	7±1	5±1	4±1

***p < 0.05 vs. P1, $p < 0.05 vs. control series.**

Urine osmolality in the absence of AVP is substantially lower. Remarkable is that with omission of urea in the infusion liquid urine osmolality starts in the normal range but declines significantly to a value that is closer to plasma osmolality towards the end (figure 4.17, table 4.10).

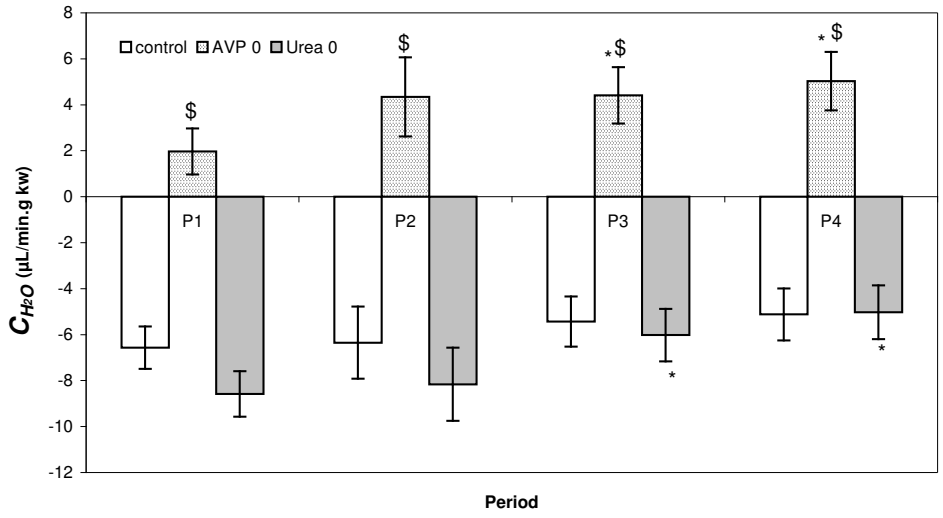

Figure 4.18: Free water clearance (C_{H_2O}) under regular conditions and in the absence of arginine vasopressin or urea. Values are estimated means ± SE. A positive value indicates that the kidney is able to produce diluted urine, while a negative value represents the concentrating ability of the kidney. As such the C_{H_2O} can be an indication for the amount of work that the kidney has to perform. *p<0.05 vs. P1, $ p<0.05 vs. control series.

4.3.10 Histology

At the end of the perfusion a slight dilatation of tubules was observed in the histological preparations. There were also indications of the presence of bacteria.

4.4 Discussion

A situation of metabolic acidosis is observed in the animals in vivo (table 4.1). The reason for this acidosis remained unclear and was surprising since the animals had a healthy appearance. It could be due to the handling of these animals that might have led to a reflex and sudden decrease of blood pressure and panting.

4.4.1 Renal blood flow and GFR

Referring to figure 3.5 in the chapter on materials and methods, it is remarkable that renal blood flow immediately at the start of the experiments was generally high. Probably this was due to a reactive hyperemia after the short period (3 – 5 minutes) of ischemia during

the process of connecting the arterial and venous lines to the respective vessels as has been demonstrated in the past (Lyrdal and Olin, 1975). Hereafter the blood flow fell within a few minutes and increased progressively thereafter.

Compared to the data in vivo, we can conclude that the RBF and GFR are close to the in vivo values of rabbits, 2.0 – 2.6 ml/min.g kw and 200 – 300 µL/min.g kw, respectively. Kozma and co-authors report a PAH-clearance of 60 ml/min for both kidneys, which is similar to the renal blood flow we are reporting. They report a 3.2 ml/min.kg creatinine clearance and 7.0 ml/min.kg inulin clearance (Kozma et al., 1974). Sejersted measured GFR's ranging from 2.9 to 4.0 ml/kidney, which is similar to our estimation of GFR (Sejersted, 1977). Values of GFR ranging from 4 – 5 ml/min for both kidneys are also in the same range of our estimations and were reported by Wong and coworkers (Wong et al., 1986). It is worthwhile to mention that in their study the rabbits had a rather high arterial blood pressure (120 mmHg). Bankir and coworkers reported a renal blood flow of 20 – 30 ml/min.kidney that is comparable to the values we are reporting (Bankir et al., 1979). However, some studies reported substantial higher blood flows (123 ml/min for both kidneys (Warren and Leidingham, 1975a) and 60 –100 ml/min.kidney (Eide et al., 1973)). The kidneys used in our experiments had an average weight of 12 – 15 grams. Kidney weight was determined immediately after perfusion. Possible increase of interstitial volume could lead to slight underestimation of our values, due to the higher (wet) weight.
Since GFR was estimated as creatinine clearance and creatinine is subjected to tubular secretion, an overestimation of the GFR can be expected (Poola et al., 2002). However the same creatinine analysis was used in a recent comparable study (Arnaud et al., 1998). On the contrary Kozma and co-authors reported a creatinine clearance (3.2 ml/min.kg) that was substantially lower than the inulin clearance (7.0 ml/min.kg) in rabbits (Kozma et al., 1974).

4.4.1.1 General aspects

There is a linear rise in renal blood flow throughout the experiments. It seems logical to assume that during surgery and exsanguination, vasoconstrictor substances will appear in the rabbit's blood (Lyrdal, 1975). Among these catecholamines and angiotensin are good candidates. Experiments are started with a certain amount of blood of the same rabbit, most probably in the presence of the above mentioned vasoconstrictive factors that are

being eliminated during the experiments. The presence of vasoconstrictive factors in the blood can be demonstrated by sudden administration of a small amount of the collected blood to the artificial circulating system, which elicits an intense vasoconstriction in the isolated kidney. This constriction can be avoided by gentle and slow infusion of the blood.

In the general introduction we have pointed out that norepinephrine as well as angiotensin cause a certain degree of vasoconstriction, especially in the efferent arteriole (Pelayo et al., 1983; Pelayo, 1988; Edwards and Trizna, 1988; DiBona and Kopp, 2000). It has also been reported that denervation of kidneys does not always alter GFR (Stella and Zanchetti, 1977; Pelayo et al., 1984). Thus at the start of the experiment a vasodilatation would be expected with isolated denervated preparations, because the innervation is probably an important factor that determines the modulation of the microcirculation (afferent and efferent arteriole) of the rabbit kidney, whereas angiotensin affects more the efferent arteriole.
Furthermore it has been described that in isolated kidney preparations the sensitivity of smooth muscle cells to vasoconstrictive factors decreases in time (Bullivant, 1978). Although this observation was more pronounced in isolated rat kidneys perfused with both protein and cell free artificial solutions, it could also play a role in isolated kidneys perfused with blood and it might be due to the absence or metabolism of an unknown substrate in the blood that is not replaced sufficiently during perfusion.
Another factor that possibly contributes to the rise in RBF could be the rather low potassium concentration. The low plasma potassium could bring vascular smooth muscle cells in a certain degree of hyperpolarisation and hence in a state of relative relaxation (Bolton et al., 1999; Hall and Guyton, 2000). However, the plasma values of potassium in the conscious rabbits (table 4.1) and of in vivo studies from the literature do not differ much from our in vitro values (Kozma et al., 1974; Sejersted, 1977; Wong et al., 1986).
One group of investigators suggested that a high glucose concentration might compromise endothelial dependent vasorelaxation (Affonso et al., 2003). In our experiments we start with a rather high glucose concentration that declines to a more physiological value towards the end.

Progressive dilatation of the efferent arteriole could lead to a decrease in GFR due to a decrease in hydrostatic pressure in the glomerular capillaries and is in accordance with our observation that GFR decreases towards the end of the experiments. The increase in RBF and

the decrease in GFR make that the filtration fraction decreases from 21% to 13%, as can be seen in figure 4.9.

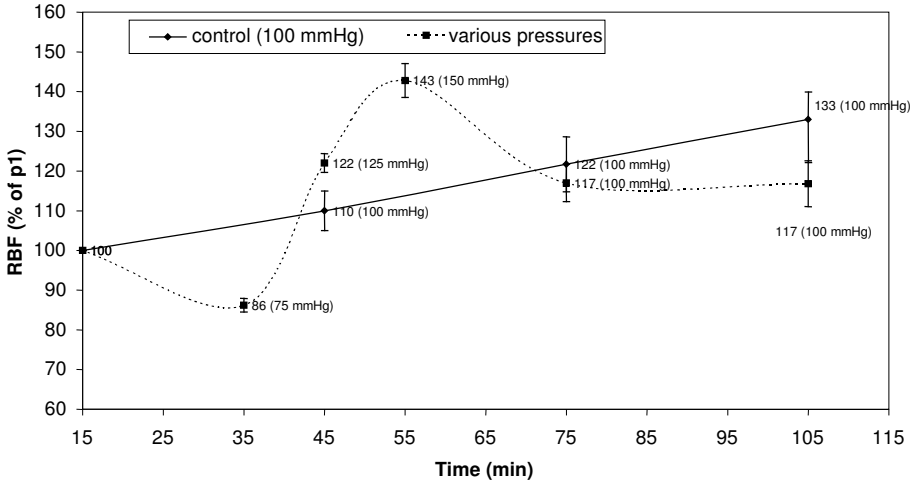

Figure 4.19: Real time plot of renal blood flow as expressed in % of P1 (15 min).

4.4.1.2 Autoregulation of RBF and GFR

At first sight we might derive from figures 4.3 and 4.4 that there is obviously no autoregulation of renal blood flow in the isolated rabbit kidney perfused with autologous blood. A progressive rise in RBF is observed during the entire experiment. In the experiments performed with different perfusion pressures, the pressures were changed only in P2, which means that when the pressure was set at 75 mmHg in the first part of P2, the resulting RBF could only be compared with the RBF of P2 in control experiments. It seemed more efficient to plot the RBF of the control experiments as well as the experiments performed at different perfusion pressures in real time, i.e. the exact time at which the RBF was reported. This yielded the graphs in figure 4.19 in which the increase or decrease of RBF is expressed as a percentage of the first period, resulting in a linear equation of RBF in real time during control condition ($y = 0.37x + 94$, $R^2 = 0.999$)s. This means that at 35 min, which corresponds to the time at which the blood flow at a perfusion pressure of 75 mmHg was reported, the estimated RBF is about 106% of P1 in the control experiments. Since RBF with a perfusion pressure of 75 mmHg is about 86% of the RBF in P1, it can be concluded that a decrease of 25% in perfusion pressure leads to a decrease of only 20% in RBF.

Similarly, a rise of 25% in perfusion pressure leads only to a rise of 12% (at 45 minutes) in RBF and an increase of 50% in perfusion pressure to a rise of (only) 29% in RBF (55 minutes).

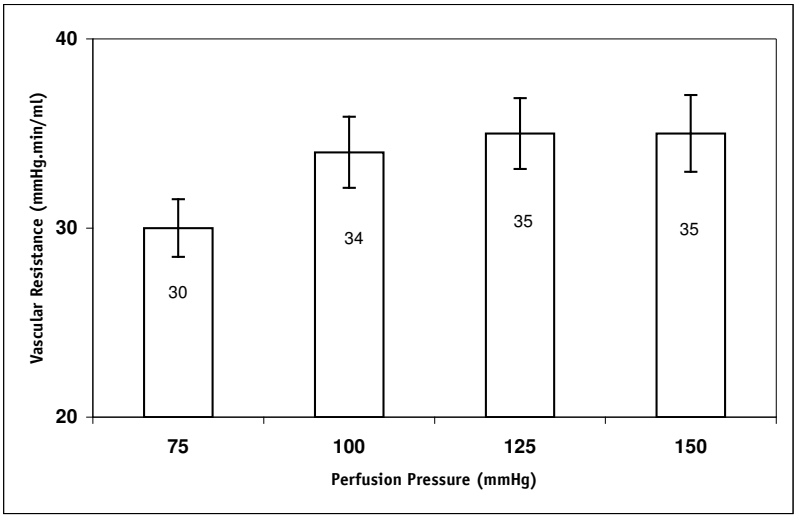

Figure 4.20: Vascular resistance with different perfusion pressures. Mean value is presented in the graph.

These figures obviously indicate that there is still some degree of autoregulation of RBF in this isolated preparation, although it is small. To accentuate these findings, renal vascular resistance was plotted against the perfusion pressure in figure 4.20. A perfusion pressure of 75 mmHg revealed a decrease in renal vascular resistance, while at perfusion pressures of 125 mmHg and 150 mmHg there was a slight increase of resistance. Taking the elastic structure of blood vessels into account, this observation is opposite to what was expected. A decrease in perfusion pressure should have allowed these vessels to shrink, meaning that resistance should increase. An increase in perfusion pressure should have led to a dilatation of the vessels with a resulting decrease of resistance, but instead a slight increase is observed. It thus appears that the vessels in this preparation do not react passively to the perfusion pressure, but actively try to counteract the consequences of changes in perfusion pressure. This might indicate the presence of some degree of autoregulation of the renal blood flow i.e. a fast myogenic mechanism.

GFR is also autoregulated and when a certain time of adaptation is allowed, it seems more complete than the autoregulation of the RBF as can be seen in figures 4.5 and 4.6. Contrary

to renal blood flow, GFR with a constantly increased perfusion pressure (figure 6), returns to control value in the second part of P2. This might indicate that the tubuloglomerular feedback is involved in this reaction. The TGF might not be an immediate mechanism, but takes some time before its effect is manifest. After restoring perfusion pressure to the initial value of 100 mmHg, following a perfusion pressure of 150 mmHg, there is very little urine flow in P3 and P4, and GFR remains low. On the other hand in the series with a constantly higher perfusion pressure of 125 mmHg in P2, GFR declines after restoration to the initial pressure of 100 mmHg, but shows a tendency to increase again to the control value in P4. These results reveal that a high perfusion pressure of 150 mmHg might be damaging the kidney. It is also possible, that the high urine flow during perfusion pressures of 125 and 150 mmHg (figure 4.10) is not enough compensated for, resulting in a higher colloid osmotic pressure of the perfusate and consequently lower filtration rate (Dworkin and Brenner, 2000; Maddox and Brenner, 2000). In chapter one it has been emphasized that in some studies colloid osmotic pressure of the perfusate in isolated perfused systems is important: by increasing it an isolated non-filtering kidney is produced (Maack, 1980).
Ott and Vari have shown that the range in which we tested the autoregulation is also the range in which the rabbit autoregulates in vivo (Ott and Vari, 1979). Moreover it has been shown that isolated kidney preparations do not autoregulate at all when perfused with cell free and protein free media (Gleim et al., 1984) and that the degree of autoregulation can be improved by adding albumins and red blood cells to the media. In fact, the degree of autoregulation rises with the hematocrit and although the presence of blood cells and protein do improve autoregulation of RBF and GFR, it is still not complete in the isolated preparations (Little and Cohen, 1974; Bullivant, 1978; Brandani Pacini and Bocci, 1983; Lieberthal et al., 1987).

Probably we should not underestimate the adrenergic influence in vivo. Autoregulation studies have been performed on denervated kidneys in vivo, but in most cases the adrenal medulla was still intact, which makes it difficult to rule out the effect of catecholamines. In fact, it has recently been shown in rabbits, that control of the RBF around mean levels is likely to be dependent on the presence of renal sympathetic nerves (Barrett et al., 2001). In addition it is very difficult to interpret physiological control mechanisms of renal blood flow in animals under general anesthesia (Warren and Leidingham, 1975b).

In a recent study Eppel and coworkers demonstrated the presence of autoregulatory responses in medullary blood flow of rabbit kidneys. However with the method they used, they were not able to measure total medullary blood flow, hence they could not exclude recruitment of vessels (Eppel et al., 2003).
On the contrary, Correia and coworkers found an almost fourfold increase of RBF when renal arterial pressure was increased from 65 mmHg to 160 mmHg in an in vivo study performed on rabbits, suggesting total absence of autoregulation of renal blood flow (Correia et al., 2002).

4.4.1.3 The effects of AVP and urea on RBF and GFR

Omission of arginine vasopressin from the infusion solution did not affect GFR significantly. Contrary to what is suggested in the literature about the effect of AVP on rat kidneys, in the absence of AVP we observe a tendency of RBF to increase (Bardoux et al., 1999; Roald et al., 2000). Iversen and Arendshorst (Iversen and Arendshorst, 1998) found that AVP stimulates calcium entry in smooth muscle cells of preglomerular arterioles, which is more in accordance with our observations of decreased RBF. However we observe a tendency for the GFR to be lower in the absence of AVP.

According to Bankir and co-workers, a low urea load would lead to a higher NaCl delivery at the macula densa and hence GFR should decrease due to the tubuloglomerular feedback (TGF) (Bankir et al., 1996; Bankir and Trinh-Trang-Tan, 2000). In contrast to this proposal, in the present study there appears to be a tendency for the GFR to be higher at the start and then it declines faster in comparison with the other two series of experiments. The absence of a significant lower GFR might be due to the fact that not only the NaCl is less reabsorbed, but also the water reabsorption has decreased, keeping the eventual NaCl concentration at the macula densa constant. But this is merely an assumption. On this isolated preparation AVP and/or urea apparently do not influence glomerular filtration rate in a significant way.

A problem that we are observing in almost all the experiments is the steady decline of GFR in the fourth period. We must admit that the mean perfusion pressure is somewhat higher than the in vivo blood pressure (85-90 mmHg) (Kozma et al., 1974; Sejersted, 1977; Wong et al., 1986) and that this higher perfusion pressure could perhaps lead to a constant

higher production of adenosine by the macula densa with resulting decrease in GFR (chapter 5). It could also be due to a regression of the vitality of the organ. In pilot experiments we were not able to perfuse these kidneys one hour longer than the regular two and a half hours.

In conclusion it can be said that this preparation does show some degree of autoregulation of RBF and GFR, but not as complete as studies in vivo. On the other hand this partial autoregulation can be elicited by the mechanism through which the TGF is exerting its effects (constriction of the afferent arteriole and dilatation of the efferent arteriole) in the rabbit kidney (Weihprecht et al., 1992; Schnermann, 1998; Ren et al., 2001).

4.4.2 Sodium and water

Fractional excretion of sodium and water remains relatively constant under regular perfusion conditions, despite a substantial and significant decrease in GFR and hence in filtered sodium load. This means that the glomerulotubular balance remains intact throughout the entire experiment and probably dominates the tubuloglomerular feedback response, which would have led to a constant absolute excretion rather than a constant fractional excretion. As we have seen earlier, TGF keeps the salt delivery to the macula densa constant.
We have already seen in the preceding paragraphs that autoregulation in this preparation is not complete, thus one might expect that GTB dominates the TGF.
On the other hand, fractional sodium excretion is somewhat higher in this isolated preparation (2-5%), compared to the fractional excretion in conscious animals. A mean fractional excretion of 1.3% for the conscious animals, used in our experiments, was calculated. Sejersted reported a value of less than 1% in anaesthetized rabbits, but with a perfusion pressure of 76±4 mmHg (Sejersted, 1977). On the other hand Wong et al reported a fractional excretion of 3 – 4% in euvolemic anaesthetized rabbits. Blood pressure was rather high in these animals and reached values of about 120 mmHg (Wong et al., 1986).
The higher FE_{Na} can be the result of the denervation, since sympathetic stimulation increases reabsorption of sodium in the proximal tubule and thick ascending limb of Henle and denervated feline kidneys exhibited an absolute increase of sodium excretion (Stella and Zanchetti, 1977; Bailly et al., 1990; DiBona and Kopp, 2000; Feraille and Doucet, 2001).

It might also be the result of a somewhat higher perfusion pressure, resulting in a slight degree of pressure natriuresis, for mean arterial pressure in the anaesthetized rabbits was slightly lower (80 – 90 mmHg). Sejertsed found the lower fractional excretion with a lower perfusion pressure (76 mmHg), while Wong and co-workers reported the higher value with higher blood pressure (120 mmHg) (Sejersted, 1977; Wong et al., 1986). As we will see further in the text, the preparation used in the present study also demonstrates pressure natriuresis.

On the other hand, a wide range of values of FE_{Na} in isolated preparations have been reported and can even reach values between 20 and 30% (see table 7 in the general introduction). In recent studies Arnaud and co-workers reported renal function values of an isolated rabbit kidney perfused with homologous blood. They found a fractional sodium excretion of about 32% with a similar GFR (creatinine clearance) as in our experiments i.e. 0.29 ml/min.g kw. They diluted the blood to a hematocrit of 25%, which might already lead to a lower reabsorption because of a decreased oncotic pressure, however a higher GFR should then be expected, but this was not reported. Fractional reabsorption of glucose in their experiments was ± 91%. This indicates that no major problem at the level of the proximal tubule function should be presumed, hence the higher excretion must be explained by a defect in transport in the more distally located tubular segments (Arnaud et al., 1998, 2000).

Absence of mineralocorticoids could also be the cause of a higher excretion of sodium, but this presumption seemed unlikely, because the transtubular potassium gradient (TTKG) was rather high in the conscious animals as well as in the isolated kidney experiments.

In the conscious animals used in these experiments, this calculation results in a value of 17.9 (table 1). In P1 TTKG = 13.9 and in P4 TTKG = 9.4. A value below 7 is suggestive for hypoaldosteronism in humans (Rose, 1994). The meaning of the TTKG has been discussed in the introduction of this chapter.

Pressure diuresis is obvious in this preparation, since increases in perfusion pressure are linearly related to absolute sodium excretion and urine flow (figures 4.10 and 4.21).

No urinary production or sodium excretion is observed at a perfusion pressure of 75 mmHg. Figure 4.21 illustrates that there is no significant difference in fractional excretion of sodium at perfusion pressures between 100 and 125 mmHg, whereas a perfusion pressure of 150 mmHg leads to an obvious increase in fractional excretion of sodium and water.

Up to perfusion pressures of 125mmHg sodium excretion increases in parallel with filtered sodium load. At 150 mmHg, filtered sodium returns to normal values, while excretion is still higher than at 125 mmHg. This can not be due to a late reaction or a consequence of dead space measurements (papilla and ureter), because urine flow was higher than in control experiments. It suggests that somewhere between 125 and 150 mmHg, the kidney loses its ability to reabsorb sodium in a proportional fashion. These findings accentuate the assumption that in the normal range of perfusion pressure in this rabbit preparation the glomerular tubular balance might outweigh the tubuloglomerular feedback. Gleim and coworkers reported a linear decrease in fractional reabsorption of sodium in the same pressure interval. However, they used rat kidneys that were perfused with an artificial solution instead of blood. Clearly, these kidneys did not demonstrate GTB (Gleim et al., 1984).

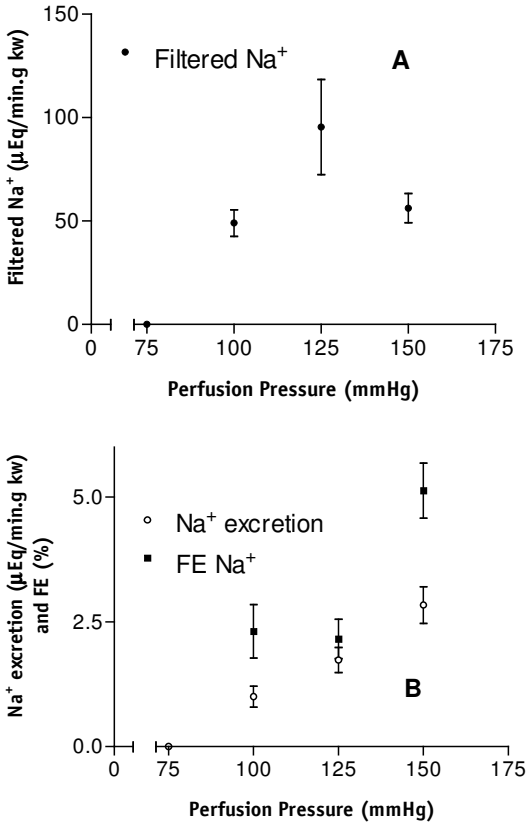

Figure 4.21: Na^+ load (A) and (fractional) excretion (B) at different perfusion pressures.

This observed pressure diuresis could be the consequence of the increased renal blood flow, which has already been reported in the chapter on hemodynamics. In spite of the conflicting results of Eppel and coworkers (Eppel et al., 2003), previous studies have shown that medullary blood vessels do not exhibit autoregulation in rat kidneys (Pallone et al., 1998; Pallone and Silldorff, 2001). As a consequence, the increase in renal blood flow might be more the consequence of an increase in renal medullary blood flow. An increase in renal medullary blood flow will wash out the osmotic gradient in the interstitium and decrease the water reabsorption in the collecting ducts, as has been explained in detail in the introductory paragraphs on renal concentrating mechanisms (chapter 1). It may also lead to an increase in interstitial hydrostatic pressure, which may counteract fluid and salt reabsorption (Garcia-Estan and Roman, 1989; Roman and Zou, 1993; Edwards et al., 2000).
In addition, the shear stress resulting from the higher perfusion pressure and blood flow might induce the release of nitric oxide in rabbit afferent arteriole (chapter 6) (Juncos et al., 1995). This nitric oxide can increase the medullary blood flow and also decrease chloride and bicarbonate reabsorption through a direct effect on the thick ascending limbs of Henle. Moreover, it has been observed that the pressure natriuresis could be blunted with inhibition of nitric oxide synthesis in rat and murine kidneys (Plato et al., 2000; Mattson and Wu, 2000; Ortiz and Garvin, 2000).

Urine flow and salt excretion are significantly higher and urine is diluted in the absence of AVP. This is complete in accordance with what should be expected and has been largely emphasized in the general introduction (DiGiovanni et al., 1994; Nielsen et al., 1995; Christensen et al., 2000; Brown and Nielsen, 2000; Agre et al., 2000; Kwon et al., 2001). In the absence of AVP, not only the osmotic water permeability of the collecting ducts decreases, but also the NaCl reabsorption in the loops of Henle and the distal parts of the nephron, increasing water and salt excretion by the kidney (Masilamani et al., 2000).

4.4.3 Glucose and phosphate

Plasma glucose level starts at a high level: 216 mg/dl (12 mM), but decreases to a more normal range in P4, 133 mg/dl (7.4 mM). Excretion of this molecule also decreases with a decrease of plasma concentration. Blood glucose levels are high, due to the glycogenolytic effect of sympathetic stimulation (Hall and Guyton, 2000). This can be a result of the surgical procedure on the animal.

Glucose excretion, like phosphate excretion, starts at a certain plasma concentration between 180 and 200 mg/dl, and increases exponentially thereafter (figures 4.13 and 4.14). There is a wide variety in excretion level after the threshold has been reached. The reason for this is most probably a variety in transporter density and regulation between the individual perfused kidneys. It is known that in rabbit proximal tubular cells, the glucose transporters are regulated by modulation of their numbers in the plasma membrane (Wright et al., 1997; Wright, 2001). Due to this phenomenon, we were not able to draw the classical titration curve. Additionally, a wide variety in filtered load of glucose, makes it more difficult. Figure 4.22 illustrates an attempt to construct the curve. It can be derived that the mean transport maximum is about 700 µg/gkw.min for the kidneys perfused in this study.

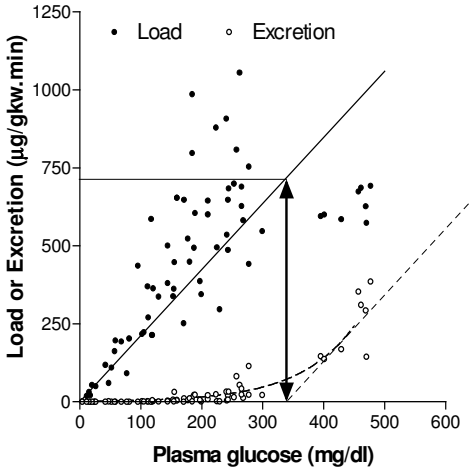

Figure 4.22: Titration curve for glucose. Estimated transport maximum is indicated by the arrow.

Fractional excretion of glucose starts at about 3% with high plasma glucose and decreases to a value below 1% in the more normal range of plasma glucose (table 7). This is lower than values with other isolated preparations (3 – 10%) (Franke et al., 1971; Schurek and Alt, 1981; Schurek and Kriz, 1985; Radermacher et al., 1999). In a comparable study, Arnaud and coworkers found a value close to 10% (Arnaud et al., 1998, 2000).

The handling of both glucose and phosphate in this preparation leads to the conclusion that the proximal tubules of this preparation function relatively well.

4.4.4 Potassium

Plasma potassium is slightly lower in our experiments, compared to the conscious animals. Both values are substantially lower than the values reported by Kozma et al (5 – 10 mM), but are comparable to the values presented by Wong et al (3.3 mM) and even higher than the values found by Sejersted (2.2 mM). This low concentration cannot be the result of a low infusion of potassium, because that would be compensated by a lower excretion of potassium as has been emphasized in the introduction of this chapter.

Fractional excretion of potassium is higher than the values reported by Wong et al and by Sejersted (25-30%) in anesthetized animals, but lower than the estimated value in the conscious animals used in these experiments (70%) (Kozma et al., 1974; Sejersted, 1977; Wong et al., 1986). On the other hand, in isolated rat kidneys perfused with Krebs Heinseleit solution, a fractional excretion of potassium reaching values between 55 and 60% has been reported (Fonteles et al., 1998). As stated earlier in this chapter the TTKG in the conscious animals as well as during the experiments is also high and although mineralocorticoids are not supplied, it might reasonably be concluded that the aldosterone content of the animals is already high and that the aldosterone concentration in the blood perfusing the isolated kidney remains high. This might be the reason for a higher excretion of potassium in the control experiments. In addition, Silva et al demonstrated that fractional excretion of potassium (FE K^+) was substantially higher in isolated perfused kidneys of potassium loaded rats (Silva et al., 1975).

Omission of AVP in the compensating infusion solution did not change the excretion of K^+. Probably the direct effect of lack of AVP and thus impaired secretion was compensated by the increased rate of urine flow, which enhances K^+ secretion. TTKG is not estimated in these experiments, because the assumption of hypertonic urine is not met (Malnic et al., 1989; Rose, 1994; Muto, 2001).

We may conclude that potassium excretion in these isolated rabbit kidneys perfused with autologous blood seems high, but this might be the result of a high mineralocorticoid activity in the animals used in these experiments. Reports on plasma potassium concentration of rabbits are conflicting, but the values used in the present experiments fall

in this wide range and are quite low. Nevertheless, urinary exretion of K^+ is well preserved, indicating that the collecting duct is functioning well.

4.4.5 Urine concentrating capacity

Absolute and fractional urea excretion are significantly higher in the absence of AVP (table 4.9 and figure 4.15). This confirms the findings that urea transport in the inner medullary collecting duct depends on AVP (Sands, 1999; Bankir and Trinh-Trang-Tan, 2000; Sands and Layton, 2000; Sands, 2000). Consequently we may conclude that the actions of AVP on this isolated rabbit kidney preparation appear to be mostly in accordance with what has been published in the literature. Hence it is not surprising to find that the preparation is able to concentrate the urine (380 – 1110 mOsm/kg) and also to dilute it (206 – 278 mOsm/kg). The hypertonic urine is on average lower in osmolality, compared to urine obtained from the conscious animals, (831 ± 81 mOsm/kg) (table 4.2). However the animals used in our experiments did not reach the urine osmolality (1100 – 1200 mOsm/kg) as found by other investigators (Barraclough et al., 1971). On the other hand it is worthwile to mention that in some series we have been able to reach these high values (chapter 5).

The in vivo urinary osmolality of the rabbits indicates that the experiments already started with a certain amount of AVP in the blood. Due to the protocol of the experiments, every blood loss was replaced by a similar amount of stored blood from the same animal. Therefore it becomes clear that, even when AVP is not administered in the compensating infusion solution, the diluting capacity of this kidney cannot be maximally achieved in the experiments performed without AVP. The minimum osmolality reached is about 233 mOsm/kg in the fourth period, compared to a plasma osmolality of 284 mOsm/kg. The fact that the plasma osmolality in P4 is lower than in P1 is explained by the high sodium excretion. The dilution of the urine indicates that the thick ascending limb of Henle is functioning properly since the diluting process largely takes place in this part of the nephron (Masilamani et al., 2000; Sands and Layton, 2000).

Figure 4.18 emphasizes the diluting and concentrating capacity of this preparation. A negative valued C_{H_2O} means that there is proportionally more water than solutes reabsorbed, whereas the opposite is true for a positive valued C_{H_2O} (Jamison and Kriz,

1982). In this figure we clearly see the negative value in the presence of AVP and the positive value in the absence of AVP. The latter increases significantly, probably as a result of a decreasing AVP concentration in the plasma.

Omission of urea in the presence of AVP decreases urine osmolality towards the end of the experiments, as can be seen in figure 4.17. This indicates that the urea reabsorption in the inner medullary collecting duct is indeed necessary for the creation of the hyperosmotic environment in the medullary interstitium, which in the end is responsible for water reabsorption out of the inner medullary collecting duct leading to concentration of the urine. Decrease of urea content in plasma (table 4.9) and thus in ultrafiltrate leads eventually to almost no urea reabsorption in the inner medullary collecting duct, as more than 50% of the filtered urea is reabsorbed in the proximal parts of the nephron (Bankir and Trinh-Trang-Tan, 2000). The resulting decrease of interstitial osmolality will withdraw less water from the thin descending limbs of the long loops of Henle, leading to a smaller increase of [NaCl] in the tip of these loops. A smaller chemical gradient for Na^+ will result in less passive NaCl reabsorption, decreasing the osmolality of the interstitium even more, leading also to a smaller osmotic driving force in the inner medulla to reabsorb water out of the collecting ducts (Masilamani et al., 2000; Sands and Layton, 2000). A significant higher absolute Na^+ excretion is not observed here, but there is a tendency for the fractional excretion of sodium to increase. The increase of fractional Na^+ excretion can also be seen in table 4.10, where the relative contribution of Na^+ to urine osmolality appears to be substantially higher in urea 0 experiments, whereas urine osmolality, which eventually depends on medullary interstitial osmolality, decreases significantly towards the end of these experiments.

It seems that the relative contribution of urea to urine osmolality is inversely proportional to that of sodium as can be seen in figure 4.23. It has been assumed that urea reabsorption in the latter part of the inner medullary collecting duct, might be secondary active via a sodium-urea cotransporter (Sands, 1999; Kato and Sands, 1999; Sands, 2000). The observed inverse relationship might be the consequence of these transporters, leading to a decreased Na^+ reabsorption with low luminal urea or an increased Na^+ reabsorption with high luminal urea content.

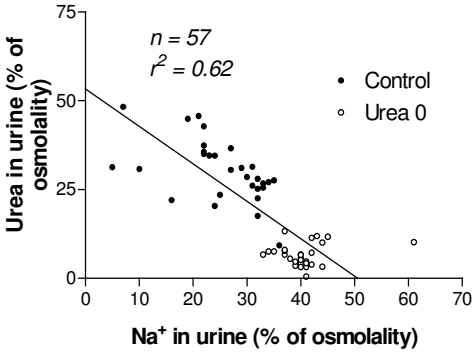

Figure 4.23: Relationship between sodium and urea in the urine.

Our observations suggest the presence of these transporters in the latter part of the inner medullary collecting duct of the rabbit, but this should be further investigated. In addition, Bankir and Trinh-Trang-Tan concluded that interruption of the urea recycling in the medulla may lead to an increase in urinary sodium concentration (Bankir and Trinh-Trang-Tan, 2000).

The necessity of urea for the reabsorption of water is further accentuated when one examines the $T^C_{H_2O}$ values in the consecutive periods in figure 4.18. With decreasing plasma urea towards the fourth period, P4, in the urea 0 experiments, the $T^C_{H_2O}$ value decreases significantly.

We may conclude that supplying the isolated perfused rabbit kidney with sufficient urea is a prerequisite for achievement of maximal urine osmolality. However urea does not affect the GFR in this preparation. Our findings are in contrast with in vivo studies, where infusion of urea did not affect urine osmolality in rabbits, contrary to what had already been found in dogs and rats (Pennell et al., 1975; Gunther and Rabinowitz, 1980).

Bankir et al found that fractional excretion of urea in rats was proportional to the urine production in the more physiological regions, but that this fractional excretion remained constant with larger urine production (Bankir and Trinh-Trang-Tan, 2000). Pooling the data of the control and AVP 0 experiments revealed about the same pattern of relation as can be seen in figure 4.16. The plateau is observed with the experiments performed without AVP. This relationship accentuates that this kidney functions in a normal fashion.

Comparison with other isolated preparations is difficult, because most of these are perfused with artificial cell free solutions and lose their capacity to concentrate the urine. This has

already been discussed in the general introduction. Arnaud and co-workers perfused rabbit kidneys with homologous blood, but they did not report any osmolality of the urine, neither the application of AVP (Arnaud et al., 1998, 2000). In summary it can be concluded that this isolated perfused preparation functions relatively well and may be a good tool, not only to investigate the proximal tubular function, but also the functions of the thick ascending limb and the collecting tubules, reflecting a normal concentrating ability and adapted K^+ excretion. The most remarkable result was the importance of urea for an optimal urine osmolality.

Chapter 5: Adenosine

Adenosine

5.1 Introduction

Adenosine is an ATP breakdown product and exerts a vasodilating effect on most systemic blood vessels in the body. In this way it contributes to metabolic control of organ perfusion i.e. matching oxygen utilisation and delivery (Saito et al., 1985; Liao and Kuo, 1997).
It is peculiar, that contrary to the majority of the systemic vessels, adenosine constricts some renal vessels and especially the afferent arteriole. In doing this adenosine decreases the utilisation of oxygen, since constriction of the afferent arteriole will lead to a decrease of tubular load and oxygen utilisation. All other renal vessels dilate when stimulated by adenosine (Hansen and Schnermann, 2003).

A_1 and A_2 receptors for adenosine have been demonstrated in rabbit kidneys in both the afferent and efferent arterioles. A_1 receptors are preferentially located in the afferent arterioles, whereas A_2 receptors in the efferent arterioles (Freissmuth et al., 1987; Ren et al., 2001). The vasoconstrictive effects can be ascribed to A_1 receptor stimulation, whereas A_2 stimulation leads to vasodilation in both rabbits and rats (Weihprecht et al., 1992; Dietrich and Steinhausen, 1993).

Furthermore adenosine has been proposed as a signalling molecule between the macula densa and the juxtglomerular apparatus in the kidney. As such it is believed that adenosine may be involved as a mediator of the tubuloglomerular feedback mechanism (see general introduction) (Brown et al., 2001). There are also indications that adenosine is involved in the control of renin secretion (Itoh et al., 1985; Weihprecht et al., 1990).

Elevation of intrarenal adenosine will result in a decreased urine flow and sodium excretion, but accompanying changes in GFR and RBF make it difficult to determine whether these effects are tubular in origin. On the contrary it has also been reported that adenosine infusions, that do not affect GFR or RBF, significantly increase urine flow and sodium excretion (Yagil, 1994).
Moreover there are reports on the diuretic consequences of adenosine A_1 receptor antagonism (Gottlieb et al., 2000). In addition, diuresis caused by A_1 receptor antagonists

seem to be without a significant increase of potassium excretion, contrary to the effect of a high ceiling diuretic as furosemide (Gottlieb et al., 2002). Reports about the effects of adenosine or its antagonists on concentrating ability of the kidney are scanty. In this chapter an attempt will be made to describe the effect(s) of adenosine and its antagonists on the isolated rabbit kidney preparation, evaluating both hemodynamic and tubular effects.

5.2 Methods and protocols

General material and methods have been described in chapter 3.

In six experiments adenosine (SIGMA) was infused at a rate of 0.1 µmol/min in P2 and in P3. In seven experiments 1 µmol of the adenosine A_1 receptor antagonist, 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) (SIGMA), was administered as a bolus dissolved in 700µl autologous plasma at the start of P2. DPCPX has a low water solubility and hence had to be administered in this way. Plasma as vehicle was also administered in a blank test. In still another series of five experiments, adenosine A_2 receptor antagonist, 1,3-dipropyl-7-methyl-8-(3,4-dimethoxystyryl)xanthine (DMPX) (SIGMA), was administered at a rate of 0.23 µmol/min during P2 - P4. The administration of adenosine, DPCPX and DMPX was coupled to the normal infusion as described for control experiments, meaning that these kidneys received regular arginine vasopressin.

5.3 Results

Renal blood flow (RBF), glomerular filtration rate (GFR) as well as the handling of sodium, potassium and osmolytes are presented in table 5.1.

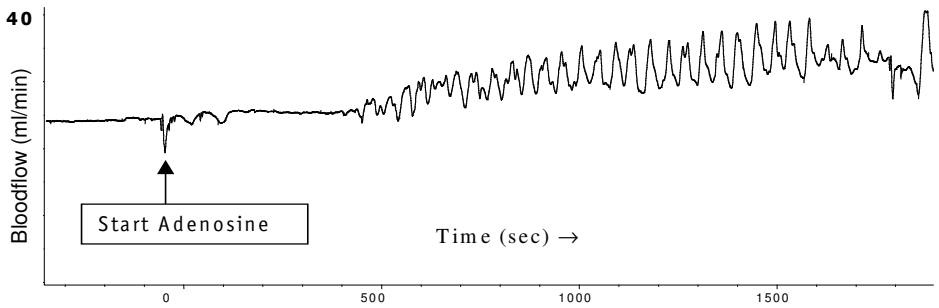

Figure 5.1: Oscillations in renal blood flow during adenosine infusion.

Table 5.1: Renal function with infusion of adenosine during P2 and P3, injection of DPCPX at the start of P2 and infusion of DMPX during P2-P4.

	Parameter	P1	P2	P3	P4
Adenosine (n=6)	RBF (ml/min.g kw)	1.9±0.2	2.9±0.3*$	3.2±0.3*	3.3±0.4*
	GFR (µl/min.g kw)	255±32	175±49*	143±35*$	127±38*
	Na^+ excr (µEq/min.g kw)	1.7±0.4$	1.1±0.4*	0.49±0.4*	0.38±0.4*
	FE Na^+ (%)	5.0±0.8$	5.3±0.8$	3.7±1.1$	2.7±1.2
	K^+ excr (µEq/min.g kw)	0.44±0.06	0.27±0.06*	0.22±0.03*$	0.20±0.04*
	Uv (µl/min.g kw)	10.0±1.6$	7.1±2.0	4.4±2.9*	4.5±3.4
	Uosm (mOsm/kg)	618±57	465±57*$	432±57*$	405±57*$
	Cosm (µl/min.g kw)	20.6±2.2$	11.7±2.7*	6.4±3.4*	6.6±3.8*
	C_{H_2O} (µl/min.g kw)	-10.6±1.1$	-4.6±1.7*	-2.0±1.2*$	-2.1±1.3*
DPCPX (n=7)	RBF (ml/min.g kw)	1.8±0.2	2.1±0.3	2.6±0.3*	3.1±0.4*
	GFR (µl/min.g kw)	355±30	345±46	307±33	242±35*
	Na^+ excr (µEq/min.g kw)	2.6±0.3$	2.9±0.3$	2.9±0.3$	2.7±0.3$
	FE Na^+ (%)	5.8±0.7$	7.0±0.7$	7.7±1.0$	8.4±1.1*$
	K^+ excr (µEq/min.g kw)	0.51±0.07	0.42±0.05	0.35±0.03*	0.28±0.04*
	Uv (µl/min.g kw)	13.1±1.5$	17.7±1.9*$	19.5±2.7*$	19.6±3.1*$
	Uosm (mOsm/kg)	606±52	446±52*$	398±52*$	371±52*$
	Cosm (µl/min.g kw)	26.9±2.0$	28.1±2.5$	28.8±3.1$	26.6±3.5$
	C_{H_2O} (µl/min.g kw)	-13.7±1.0$	-10.3±1.6*	-9.3±1.1*$	-7.0±1.2*
DMPX (n=5)	RBF (ml/min.g kw)	2.8±0.3$	2.9±0.3	3.2±0.4	3.5±0.4*
	GFR (µl/min.g kw)	409±35$	448±54$	406±39$	349±42$
	Na^+ excr (µEq/min.g kw)	1.0±0.4	1.7±0.4*	1.8±0.4*$	1.4±0.4
	FE Na^+ (%)	1.9±0.9	3.1±0.9	3.7±1.2	3.4±1.3
	K^+ excr (µEq/min.g kw)	0.45±0.07	0.68±0.07*$	0.47±0.03$	0.33±0.04
	Uv (µl/min.g kw)	3.9±1.8	7.0±2.3	9.0±3.0	8.1±3.7
	Uosm (mOsm/kg)	1160±62$	886±62*$	662±62*	578±62*
	Cosm (µl/min.g kw)	14.1±2.4	20.4±2.7*$	22.1±3.7*$	17.4±4.2
	C_{H_2O} (µl/min.g kw)	-10.2±1.2$	-13.5±1.9$	-13.1±1.3*$	-9.3±1.4$

RBF, renal blood flow; GFR, glomerular filtration rate; excr, excretion; FE, fractional excretion; Uv, urine flow; Uosm, urinary osmolality; Cosm, osmolar clearance; C_{H_2O}, free water clearance. Values are estimated mean ± SE, n = number of experiments, *p<0.05 vs. P1, $ p<0.05 vs. control series (chapter 4).

Adenosine infusion led to a significant increase of renal blood flow in P2 compared to P1 and the control series (chapter 4). The blood flow remained high and did not decrease after

cessation of the infusion. In the majority of experiments a transient oscillation of renal blood flow was observed. This pattern of RBF is demonstrated in figure 5.1. Adenosine A_1 receptor antagonist, DPCPX, elicited an extensive vasoconstriction, immediately after administration (figure 5.2), but this reaction vanished within 2 minutes. Apart from this acute reaction, DPCPX and DMPX did not affect the renal blood flow significantly.

GFR decreased with the infusion of adenosine, and this decrease became distinct in P3. DPCPX had little effect on the glomerular filtration, while DMPX tended to increase this rate, although differences were not significant and glomerular filtration rate in this series started at a higher level.

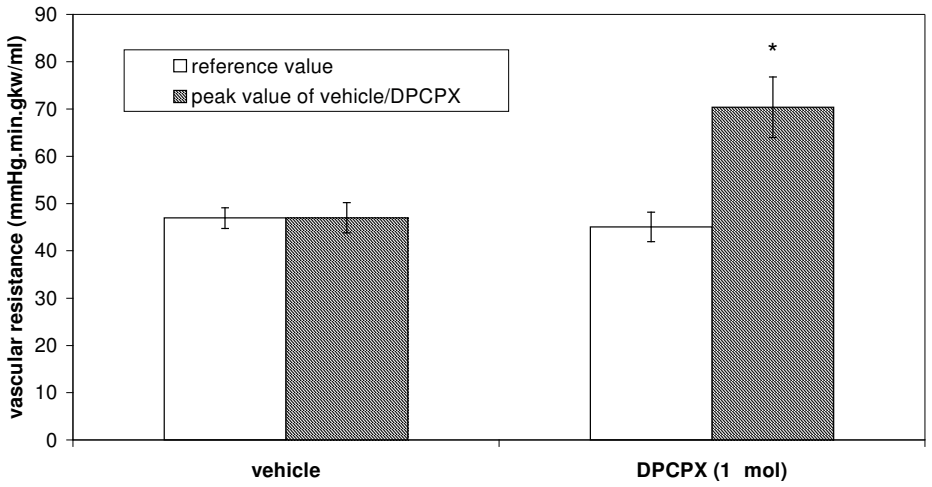

Figure 5.2: Acute effect of DPCPX on renal vascular resistance. Values are mean ± SEM, *p<0.05 vs control.

For unknown reasons, sodium excretion started at a higher level in the first two series i.e. adenosine and DPCPX, making comparison with the control series (chapter 4) difficult. In the control experiments sodium excretion tended to decrease gradually during the experiments. This same tendency can be observed with the infusion of adenosine. There was no change of sodium excretion with DPCPX, while DMPX increased this excretion. Potassium excretion decreased in the presence of adenosine, while it was not affected by DPCPX and, on the contrary, it was increased by DMPX.

Urine flow also started at a higher level with adenosine and DPCPX. Adenosine tended to decrease this flow, while DPCPX and DMPX tended to increase this. In the control experiments urine flow remained relatively constant during the four periods.
Urine osmolality decreased in all three situations. Osmotic clearance decreased with adenosine, did not change in the presence of DPCPX and increased with DMPX. Free water clearance decreased with adenosine and DPCPX and demonstrated a tendency to increase with DMPX.

5.4 Discussion

5.4.1 Hemodynamic parameters

Infusion of adenosine in our experiments produces a vasodilatation. The higher blood flow did not lead to an increase in GFR, but instead a substantial decrease of glomerular filtration was observed. This can be explained if adenosine induces an efferent arteriolar dilatation and a possible constriction of the afferent arteriole. As explained in the introductory paragraphs, this is possible in rabbit kidneys, since A_2 receptors responsible for vasodilatation are preferentially located in the efferent arteriole, whereas A_1 receptors producing vasoconstriction, are normally confined to the afferent arteriole (Hansen and Schnermann, 2003). Probably at the dose used, the vasodilatory effect prevails above the constrictory effect, hence the observed increase in renal blood flow. If adenosine is indeed the mediator of the tubuloglomerular feedback (Osswald et al., 1997; Schnermann, 2002; Vallon, 2003), then the mechanism of autoregulation of renal blood flow in rabbit kidneys is difficult to explain by this TGF, since both vasoconstriction and vasodilatation are present (chapters 1 & 4).

According to the data from the literature, administration of an A_1 adenosine receptor antagonist would produce a vasodilatation of the afferent arteriole, supposing adenosine secretion is continuous in the isolated kidney. However we observed an immediate vasoconstriction, rapidly followed by a vasodilatation bringing RBF to control values. The vasoconstriction could be due to a sudden rise in renin production, leading to an activation of angiotensin II. It has been reported that A_1 receptor blockade increases the renin production by the granular cells (Barchowsky et al., 1987; Weihprecht et al., 1990; Albinus et al., 1998). The decrease of renin production through A_1 receptor stimulation might also account for the vasodilatation, observed when adenosine is infused. However, GFR does not

change significantly. This can be explained as follows. Probably, initially A_1 receptor blockade will produce a rise in GFR, but secondly via the TGF, the adenosine production of the macula densa will increase, thereby activating more A_2 adenosine receptors and causing dilatation of the efferent arteriole with a decrease to normal GFR (Ren et al., 2001). The vasodilatation is not very obvious, because of the putative concomitant increased production of angiotensin II. No significant change in RBF and GFR under A_2 adenosine receptor blockade was observed, possibly via the direct and indirect antagonistic effects of the A_1 receptor stimulation. These results suggest that adenosine might be responsible for the tubuloglomerular feedback in the isolated rabbit kidney and that adenosine is continuously being produced in this preparation. The negative feedback character of the TGF results in an oscillating profile of the renal blood flow and in fact every single nephron has an individual oscillating GFR (Holstein-Rathlou and Marsh, 1994). Supplying the kidney with a high dose of adenosine might synchronize the oscillations of all the single nephrons, resulting in the larger macroscopic ones, seen with total RBF (figure 4.1).

5.4.2 Sodium and water

Adenosine will also affect tubular function, as well as the microcirculation. An increase in renal blood flow and a decrease in GFR must result in a drop in filtration fraction and colloid osmotic pressure in the efferent arteriole and peritubular capillaries. This should normally lead to a decreased proximal reabsorption of fluid and salt (chapters 1 & 4). On the contrary, it is observed that (fractional) excretion of sodium and water do not increase, but instead show a tendency to decrease. Moreover, the observed increase of sodium excretion in the presence of DMPX, without a significant change in GFR, supports an enhanced reabsorption of sodium by adenosine.

In studies on medullary rabbit thick ascending limbs it has been observed that high dose A_2 stimulation led to an increase of intracellular cAMP, while low dose A_1 stimulation resulted in a decrease of cAMP (Burnatowska-Hledin and Spielman, 1991). Theoretically, this could mean that A_1 receptor stimulation by low dose adenosine infusion would result in a decrease of Na^+ reabsorption, whereas A_2 receptor stimulation by high dose adenosine infusion would lead to an increase of sodium reabsorption in the thick ascending limb (Feraille and Doucet, 2001).

But it has also been demonstrated in the proximal tubules of rabbits, that A_1 stimulation led to an increase of sodium reabsorption via stimulation of the sodium bicarbonate transporter in the basolateral plasma membrane (Takeda et al., 1993). On the other hand, vasopressin mediated water reabsorption in the rat inner medullary collecting duct is inhibited by A_1 receptor stimulation (Edwards and Spielman, 1994).

Consequently A_1 receptor inhibition would lead to a decreased proximal sodium reabsorption and an increase in thick ascending limb and collecting duct reabsorption. The findings in the present study are controversial with the observations that adenosine increases urine production and sodium excretion (Yagil, 1994). With the application of DPCPX a diuretic effect is observed, while DMPX leads more to a natriuretic effect. These observations are in accordance with the literature (Gottlieb et al., 2000).

5.4.3 Potassium

Potassium excretion in this preparation is decreased in the presence of adenosine and initially increases with the application of DMPX. This can be the result of the tendency of decreased sodium excretion in the presence of adenosine and the increase of sodium excretion with DMPX (see section 1.1.5.4). This hypothesis is supported by the results of DPCPX, for in this situation there is little change in sodium and potassium excretion. A_1 receptor antagonists have been proposed as potassium sparing diuretics, but in the present study A_1 inhibition does not lead to an overwhelming increase of Na^+ as we will see with the application of diuretics in chapter 7, although there still is a substantial increase of urine flow (Gottlieb et al., 2002; Welch, 2002).

5.4.4 Concentrating ability

Table 1 reveals that adenosine infusion compromises the concentrating ability of the kidney. This is more the consequence of a decrease in solute excretion than an increase in water loss, because urine production also decreases. The decrease of the osmotic clearance and $T^C{}_{H_2O}$ further supports this assumption. The observed decrease in GFR (P3 obviously more than P2) might decrease the solute delivery to the distal parts of the nephron. In addition there is also an increase of renal blood flow. It has not been determined whether this affects the renal medullary blood flow as well, which might wash out the solute

gradients in the medullary interstitium and eventually result in a decreased urine osmolality (Pallone et al., 1998).

Moreover there is evidence available that adenosine A_1 receptor stimulation inhibits the AVP mediated increase in osmotic water permeability in the collecting ducts (Edwards and Spielman, 1994).

Adenosine A_1 and A_2 receptor blockade also decrease urine osmolality. Contrary to adenosine, this appears to be more the consequence of an increase in urine volume than a decrease of osmolytes.

It can be concluded that in this preparation adenosine induces an increase of renal blood flow, but a decrease of GFR. The concentrating capacity of the kidney is compromised.

Chapter 6: Nitric oxide

Nitric oxide

6.1 Introduction

Nitric oxide (NO), formerly known as endothelial derived relaxing factor (EDRF), normally causes vasodilatation in renal blood vessels. It elicits a dilatation in both afferent and efferent arterioles of rabbits (Edwards and Trizna, 1993; Juncos et al., 1995). Furthermore, NO induces vasodilatation of medullary vessels, which guarantees oxygen supply to inner medullary regions of the kidney (Pallone et al., 1998).

Changes in macula densa NO production may underlie the resetting of the tubuloglomerular feedback mechanism (TGF). The importance of NO to TGF resetting and the substrate dependence of NO production have both been found during changes in dietary salt (Wilcox et al., 1998). It is suggested that NO resets the efficacy of the TGF, making it possible to keep the salt balance in equilibrium during periods of high salt intake when a higher glomerular filtration is necessary (Thomson et al., 1998; Vallon, 2003).

Nitric oxide is derived from the amino acid L-arginine through the action of nitric oxide synthase (NOS) (Wu et al., 2000). Micropuncture studies of single nephrons have shown that macula densa solute reabsorption activates nitric oxide (NO) generation via neuronal-type NO synthase (nNOS). This pathway is enhanced during salt loading (Thomson et al., 1998; Wilcox et al., 1998; Welch et al., 1999; Vallon et al., 2001). Other NOS isoforms may be produced in the mesangium, and glomerular microvessels (Wilcox, 2000). Intrarenal NO generation increases also with renal perfusion pressure and this NO response is implicated in reducing renal tubular sodium reabsorption (pressure natriuresis). Low doses of NO will stimulate proximal tubular fluid reabsorption, whereas high doses may inhibit this process. NO inhibits NaCl reabsorption in the thick ascending limb of Henle's loop and in the collecting ducts (Wilcox, 2000).

Increased glomerular NO synthesis seems to play a role in the decreased renal vascular resistance observed after unilateral nephrectomy in rats (Valdivielso et al., 1999). It has been proposed that, if resetting of the TGF is not adequate, hypertension can develop (Persson et al., 2000).

Thus NO exerts effects on both hemodynamics and tubular functions of the kidneys. In this experimental setting an attempt was made to evaluate the effects of intrarenal infusion of L-arginine and the NOS inhibitor L-NAME on the function of the current preparation.

6.2 Methods and protocols

Organ preparation and general procedures have already been presented in chapter 3. In seven experiments, L-arginine as precursor and inducer of nitric oxide (NO) production, was infused during P2 at a rate of 7.2 μmol/min and in five additional experiments the nitric oxide synthase antagonist, N^{ω}-nitro-L-arginine methyl ester (L-NAME), was infused at a rate of 185 nmol/min in P2. All kidneys received the regular compensating infusion containing arginine vasopressin as well.

6.3 Results

Table 6.1: Renal function parameters with infusion of L-arginine or L-NAME during P2.

	Parameter	P1	P2	P3	P4
L-arginine (n=7)	RBF (ml/min.g kw)	2.0±0.2	2.9±0.3*	3.5±0.3*$	3.6±0.4*
	GFR (μl/min.g kw)	293±30	336±46	263±33	230±42
	Na^+ excr (μEq/min.g kw)	1.8±0.3	1.4±0.3	0.9±0.3*	0.8±0.3*
	FE Na^+ (%)	5.2±0.7$	3.7±0.7*	3.1±1.0*	3.0±1.1
	K^+ excr (μEq/min.g kw)	0.47±0.06	0.31±0.06*	0.31±0.03*	0.25±0.04*
	Uv (μl/min.g kw)	10.0±1.5$	9.6±1.9	7.1±2.7	7.2±3.1
	Uosm (mOsm/kg)	549±52	534±52$	501±53$	419±52*$
	Cosm (μl/min.g kw)	19±2$	16±3	12±3*	11±4*
	C_{H_2O} (μl/min.g kw)	-8.2±0.99	-6.7±1.6	-5.2±1.1*	-4.1±1.2*
L-NAME (n=5)	RBF (ml/min.g kw)	3.2±0.3$	2.5±0.3*	3.6±0.7$	4.4±0.4*$
	GFR (μl/min.g kw)	319±35	208±54*	234±39*	224±42*
	Na^+ excr (μEq/min.g kw)	1.7±0.4	0.7±0.4*	0.4±0.4*	0.7±0.4*
	FE Na^+ (%)	4.0±0.9	2.7±0.9	1.6±1.2*	2.5±1.3
	K^+ excr (μEq/min.g kw)	0.63±0.07	0.34±0.07*	0.30±0.03*	0.35±0.04*
	Uv (μl/min.g kw)	8.4±1.8	3.9±2.3*	4.5±3.1	7.8±3.7
	Uosm (mOsm/kg)	634±62	654±62	546±62	434±62*$
	Cosm (μl/min.g kw)	18±2	9±3*	9±4*	12±4
	C_{H_2O} (μl/min.g kw)	-9.6±1.2$	-5.0±1.9*	-4.0±1.3*	-4.0±1.4*

***p<0.05 vs. P1, $p<0.05 vs. control series (chapter 4).**

Renal blood flow, glomerular filtration rate, sodium excretion, potassium excretion and renal concentrating capacity are presented in table 6.1. Infusion of L-arginine produced an obvious increase of renal blood flow, while GFR demonstrated a slight tendency to increase. On the contrary, L-NAME produced a significant decrease of both renal blood flow and GFR

during infusion. In spite of the tendency to increase the GFR, excretion of Na^+ tended to decrease with L-arginine. Fractional excretion of sodium decreases significantly with application of L-arginine. L-NAME decreased sodium and water excretion. Potassium excretion decreased in both settings. Urine osmolality was hardly affected, while osmolar clearance (C_{osm}) and negative free water clearance ($T^C_{H_2O}$) both decreased in the presence of L-NAME.

6.4 Discussion

6.4.1 RBF and GFR

L-arginine increased renal blood flow without a significant increase in GFR. This implicates that the increase in RBF was a result of dilatation in the afferent as well as the efferent arteriole. Taking the slight tendency to increase the GFR into account, as well as the decrease of GFR and RBF with L-NAME, it points to a mechanism that influences more the afferent arteriole than the efferent arteriole. In rats it has been reported that blockade of NO synthesis led to a decrease of GFR and renal plasma flow (Tolins and Raij, 1991). In addition it has been demonstrated that NO, derived from eNOS and iNOS in the thick ascending limb of Henle's loop, inhibits the tubuloglomerular feedback, explaining the rise in RBF and tendency of GFR to increase (Wang et al., 2002). Systemic inhibition of NO synthesis in human subjects has been reported to decrease GFR, renal plasma flow and excretion of water and salt (Bech et al., 1996).

On the other hand, supplying rabbit kidneys in vivo with an NO donor without affecting perfusion pressure hardly changed RBF, GFR and excretion of water and salt (Adachi et al., 1997).

6.4.2 Na^+,K^+ and H_2O

Contrary to what might be expected, the increase of renal blood flow and the tendency of GFR to augment did not stimulate sodium excretion, but instead fractional excretion of Na^+ decreased, suggesting enhancement of salt reabsorption. Potassium excretion also decreased, probably as a result of a decreased sodium delivery at the distal sites of nephron (chapter 4). However it has been reported that infusion of L-arginine causes increased diuresis (Costa et al., 2001) and that NO inhibits chloride and bicarbonate reabsorption in the thick ascending limb of Henle's loop (Plato et al., 2000; Ortiz and Garvin, 2000; Ortiz et al., 2001). Moreover, it has also been demonstrated that inhibition of NO synthesis

decreases renal medullary blood flow and enhances salt retention (Mattson and Wu, 2000). These arguments favour the observations in the present study that infusion of L-NAME led to a decrease of sodium and water excretion with a resulting decrease also in potassium excretion.

On the contrary it has been demonstrated that NO increases proximal sodium reabsorption, but this appeared to depend on sympathetic stimulation, which is absent in our preparation (Wu et al., 1999; Wang et al., 2000). However, in a more recent study it was demonstrated that this upregulation of water and salt transport did not have to depend on neuronal stimulation (Wang, 2002).

6.4.3 Concentrating ability

L-arginine and L-NAME do not seem to affect renal concentrating ability in these experimental settings, although the RBF and GFR are both affected. Besides, it would be expected that with increasing (medullary?) blood flow, water excretion increases with a resulting decrease in urine osmolality (Pallone and Silldorff, 2001). Additionally, it has been demonstrated in several studies, that NO inhibits Na^+ and HCO_3^- reabsorption in the TAL (Bailly, 1998; Lu et al., 1998; Garvin and Hong, 1999; Ortiz and Garvin, 2000; Capasso et al., 2002) and induces a decrease of water and salt permeability of the rat cortical collecting duct, that is normally induced by AVP (Garcia et al., 1996). These studies may explain the decrease of water excretion as observed with infusion of L-NAME. This correlates with the observed decrease of osmolar and free water clearance in the present study.

It can thus be concluded that in accordance with the majority of published studies, NO leads to vasodilatation in isolated rabbit kidneys. The effects of L-arginine on salt and water excretion in this preparation are however controversial, but this controversy also exists in the available literature. On the other hand, these variations may be the result of a possible lower NO generation, when infusing specific amounts of L-arginine (Wilcox, 2000).

Chapter 7: Diuretics

Diuretics

7.1 Introduction

Edematous disorders and hypertension are disorders amongst others in which diuretics are commonly used as therapy. Various classes of diuretic drugs exist, each acting on a different molecular substrate. Moreover, the development of these diuretics has aided in the discovery of specific sodium transporters along the nephron and disorders due to genetic defects of these proteins. As an exception, osmotic diuretics do not have a specific protein substrate to exert their effects (Okusa and Ellison, 2000). In this chapter, two kinds of diuretics will be evaluated on their effects on the present preparation: mannitol, as an osmotic diuretic, and furosemide, as a loop diuretic.

Osmotic diuretics are substances that are freely filtered at the glomerulus, but are poorly reabsorbed. The effect of drugs in this group of diuretics depends entirely on the osmotic pressure exerted by the drug molecules in solution and not on interaction with transport proteins or enzymes.

The major renal actions of mannitol are:

1. variable effects on renal blood flow
2. variable effects on GFR
3. natriuretic and diuretic action due to inhibition of tubular reabsorption of water and salt (decrease of proximal tubule fluid reabsorption)
4. increase in renal excretion of other ions, e.g. potassium
5. depression of renal concentrating ability

(Barraclough et al., 1970; Kiil et al., 1971; Lang, 1987; Leyssac et al., 1990; Better et al., 1997; Leyssac et al., 2000; Okusa and Ellison, 2000)

Loop diuretics, like furosemide, have the highest natriuretic and diuretic potency. They can increase Na excretion up to 25% of the filtered load. In contrast to osmotic diuretics, the urine always becomes isosmotic with plasma. Furosemide binds reversibly to the Na-K-2Cl carrier on the apical plasma membrane of the thick ascending limb of Henle cells. As a consequence, it reduces or abolishes NaCl reabsorption in this nephron segment and leads to a decreased interstitial hypertonicity and thus to a reduced water reabsorption by the collecting ducts (Okusa and Ellison, 2000).

Summarising renal effects of furosemide:

1. variable effects on renal blood flow

2. variable effects on GFR
3. very high natriuretic and diuretic activity
4. elimination of renal concentrating and diluting ability
5. strong kaliuretic

(Bowman et al., 1973; Scherzer et al., 1987; Shirley et al., 1992; Gimenez et al., 1998; Romano et al., 1999; Okusa and Ellison, 2000)

7.2 Methods and Protocols

General materials and methods are described in chapter 3. In a series of five experiments the osmotic diuretic mannitol (15%, 0.1 ml/min) was infused during periods P2 – P4. In another series of five experiments, the loop diuretic furosemide was infused at a rate of 6 µg/min during P2 and at a rate of 180 µg/min during P3 and P4. All kidneys received the regular infusion of glucose, urea, electrolytes and AVP.

7.3 Results

The acquired data are presented in table 7.1. Mannitol causes an early increase in renal blood flow, while an increase of renal blood flow is only observed with the higher infusion dose of furosemide. GFR remains stable with mannitol, but is significantly depressed in the presence of furosemide. Sodium and water excretion are increased in both experimental settings. Fractional excretion of sodium increases 4 – 5 times. Potassium excretion does not increase and on the contrary decreases in the presence of mannitol. However, plasma potassium decreases in both settings. Urine osmolality decreases in the presence of mannitol, but remains relatively high. On the other hand, furosemide lowers urine osmolality to almost plasma values. A dramatic decrease of urine concentrating capacity in this setting is also expressed in the fast decline of free water clearance by furosemide, whereas this drop is less and is only seen in the last perfusion period with mannitol. Plasma osmolality increases progressively in the presence of mannitol, while it becomes slightly lower during furosemide infusion. Osmolar clearance increases with both regiments in a comparable fashion.

Table 7.1: Renal function parameters with mannitol or furosemide infusion during P2 – P4.

	Parameter	P1	P2	P3	P4
Mannitol (n=5)	RBF (ml/min.g kw)	2.5±0.3	3.0±0.3*	3.2±0.4*	3.4±0.4*
	GFR (µl/min.g kw)	298±35	306±54	231±39	203±42*
	Na^+ excr (µEq/min.g kw)	2.3±0.4$	2.7±0.4$	4.5±0.4*$	6.5±0.4*$
	FE Na^+ (%)	5.2±0.9$	6.7±0.9$	14.0±1.2$	21.0±1.3$
	PK^+ (mEq/L)	2.8±0.3	2.2±0.2*$	1.8±0.2*$	1.7±0.3*$
	FE K^+ (%)	68±9	67±9	68±9$	82±9$
	K^+ excr (µEq/min.g kw)	0.51±0.07	0.43±0.07	0.27±0.03*	0.27±0.04*
	Uv (µl/min.g kw)	12±2$	26±2*$	39±3*$	50±4*$
	Posm (mOsm/kg)	295±5	304±6*$	335±6*$	360±6*$
	Uosm (mOsm/kg)	608±62	466±62*	424±62*	434±62*
	Cosm (µl/min.g kw)	25±2$	40±3*	50±4*	58±4*
	C_{H_2O} (µl/min.g kw)	-13±1$	-14±2$	-10±1$	-8±1*
Furosemide (n=5)	RBF (ml/min.g kw)	2.5±0.3	2.8±0.3	3.3±0.4*	4.0±0.4*$
	GFR (µl/min.g kw)	395±35$	310±54*	257±39*	269±42*
	Na^+ excr (µEq/min.g kw)	1.9±0.4	2.5±0.4*$	5.1±0.4*$	6.0±0.4*$
	FE Na^+ (%)	3.7±0.85	6.3±0.86$	18±1.3$	21±1.4$
	PK^+ (mEq/L)	2.5±0.26	2.3±0.21$	1.9±0.23*$	1.7±0.31*$
	FE K^+ (%)	51±8.7	71±8.7*$	103±9.1*$	93±9.4*$
	K^+ excr (µEq/min.g kw)	0.49±0.07	0.51±0.07	0.43±0.04$	0.33±0.07
	Uv (µl/min.g kw)	9±2	17±2*$	40±3*$	45±4*$
	Posm (mOsm/kg)	292±5	280±6*	280±6*	282±6*
	Uosm (mOsm/kg)	686±62	466±62*	303±65*	298±67*
	Cosm (µl/min.g kw)	21±2$	26±3	43±4*	48±5*
	C_{H_2O} (µl/min.g kw)	-12±1$	-9±2	-5±1*	-5±2*

Values are estimated means ± SE. RBF, renal blood flow; GFR, glomerular filtration rate; excr, excretion; P, plasma; FE, fractional excretion; Uv, urine flow; osm, osmolality; n, number of kidneys. *p<0.05 vs. P1, $ p<0.05 vs. control series (chapter 4).

7.4 Discussion

7.4.1 Renal blood flow

The increase in renal blood flow with mannitol is probably not the result of an increased demand of oxygen, since sodium reabsorption is not increased. On the contrary, absolute and fractional sodium excretion are increased. Moreover there is a tendency to decrease

$T^{C}_{H_2O}$. There is also evidence available that in dog kidneys mannitol does not increase the metabolic rate (Kiil et al., 1971). In an isolated perfused rat kidney Lindström and co-workers found a decrease of renal vascular resistance. They also found an increase of intratubular and peritubular capillary lumen which might indicate the possibility of cellular shrinkage, since there was also a tendency of increased interstitial volume. On the other hand they observed an increase of glomerular cross-section area, which might argue against a decrease of cellular volume with infusion of mannitol. It must be mentioned that they perfused the kidneys at a temperature of 8°C (Lindstrom et al., 1999). In an in vivo study on rats, however, a decrease of renal blood flow was observed during mannitol administration (Leyssac et al., 2000) whereas no change was reported in another study (Buerkert et al., 1981).

In isolated rat kidneys, furosemide did not change renal vascular resistance significantly (Gimenez et al., 1998) and this lack of change in renal blood flow was also observed in cats (Stella and Zanchetti, 1977). In this isolated rabbit kidney there is a slight increase of renal blood flow at the end of the experiments. The absence of vascular reactions is peculiar, since furosemide normally blocks the tubuloglomerular feedback (Okusa and Ellison, 2000) and hence a dilatation is expected. Probably the amount of furosemide that reaches the macula densa is initially low, since a late increase of RBF is observed with the higher concentration of furosemide infused in P3 and P4.

7.4.2 Glomerular filtration rate

The evolution of GFR remains comparable with the control series when mannitol is infused: it does not change significantly during P2 and P3. In vivo measurements of GFR in rats infused with mannitol revealed a decrease (Leyssac et al., 1990; Leyssac et al., 2000) whereas, it was demonstrated that mannitol increased the GFR to a value that was 50% higher than the initial value in an isolated rat kidney perfused at 8°C (Lindstrom et al., 1999).

In the presence of furosemide, the GFR decreased already in P2 and remained lower during the course of the experiments. On the other hand GFR was not affected by furosemide in some studies performed on rabbits (Pichette et al., 1999) and rats (Shirley et al., 1992). However, some other studies performed on isolated preparations, as well as in vivo experiments on cats and rats, revealed a significant decrease of GFR (Bowman et al., 1973;

Stella and Zanchetti, 1977; Gimenez et al., 1998), while another study reported an increase of GFR on long term use (7 days) (Scherzer et al., 1987). In human subjects suffering from congestive heart failure, GFR was reduced after administration of furosemide (Gottlieb et al., 2002). The decreased GFR in our experiments supports the previous hypothesis that the decrease of salt reabsorption in the thick ascending limb of Henle's loop might surpass the blocking effect at the macula densa location and thus elicit the TGF mechanism.

7.4.3 Potassium

As expected, both mannitol and furosemide reveal an obvious natriuretic and diuretic effect in this preparation, but despite this natriuresis, absolute potassium excretion is not increased in the present study. Taking the significantly decreased plasma potassium values into account, leading to a relatively low potassium filtrate, it becomes clear that furosemide still exhibits a higher fractional excretion of potassium and even net K^+ secretion in P3. This is complete in accordance with the available literature, as described in the introductory paragraphs of this chapter. For obscure reasons, absolute and fractional excretion of potassium are not increased with mannitol. It might be due to the decreasing plasma [K^+], which depresses absolute K^+ excretion (chapter 1). However, these same low [K^+] values are observed with furosemide. Clearly in this preparation, furosemide exhibits a high kaliuretic effect, while potassium excretion is not obviously increased in the presence of mannitol.

7.4.4 Urine osmolality

The effect of mannitol on the urine osmolality is clearly a consequence of a higher water excretion, since osmotic clearance increases as well as urine flow. $T^C_{H_2O}$ remains high and saturates or decreases only in the last perfusion period. This has also been reported in studies performed in vivo (Barraclough et al., 1970; Gunther and Rabinowitz, 1980). In other words, urine osmolality decreases, but the kidney still ensures the excretion of osmolytes in a relatively small amount of water, which indicates that the transport of NaCl along the TAL has increased with the elevated salt load from the proximal tubule, resulting in a constant concentration of NaCl at the macula densa. Therefore the GFR remains constant.

On the contrary, furosemide increases the salt excretion, by blocking the salt reabsorption in the thick ascending limb of Henle and thus the single effect in the countercurrent

multiplication system (chapter 1) (Davis and Briggs, 1987). The interstitial salt concentration will decrease, rendering the urine nearly isotonic. There are reports stating that during furosemide administration the urine approaches isosmolality, irrespective of the baseline Uosm (Shirley et al., 1992; Romano et al., 1999).

The results of this study suggest that influences of mannitol and furosemide on this preparation are comparable with studies performed in vivo and in vitro.

Chapter 8: Beneficial effect of mannitol on renal function during and after hypoxic stress

Beneficial effect of mannitol on renal function during and after hypoxic stress

8.1 Introduction

Tubular injury plays a central role in the pathophysiology of ischemic acute renal failure. The reduction of GFR observed in these conditions can be due to either tubular obstruction or tubular back leak of glomerular filtrate or both. The obstruction of tubular flow can be understood when evaluating alterations of cellular function in hypoxic conditions. Oxygen deprivation leads to a rapid degradation of cellular ATP. In the end the balance between oxygen demand and supply will determine the degree of injury to the cells, however, one immediate effect of ATP depletion is cell swelling (Brady et al., 2000).

It is known that the maintenance of a constant cell volume requires the utilisation of energy to drive the Na^+-K^+ ATPase in order to establish and maintain ion gradients across the cell membranes. Inhibition of this pump during ischemia will eventually lead to potassium loss and a gain of NaCl followed by water across the cell membrane, resulting in a rapid increase of cell volume. In normoxic conditions cells that swell will regain their original cell volume by the so-called regulatory volume decrease (RVD): volume regulated channels will open and induce a loss of KCl. Concomitantly water will follow.

The characteristic RVD will not occur in conditions of ATP depletion, because K^+ might already be lost in the early phase of metabolic inhibition and not be available anymore as a solute for efflux, while NaCl replaces KCl in the cytosol. However it was shown that the cells stop swelling when exposed to metabolic inhibition. Part of it might be explained by the intracellular acidification due to the ATP hydrolysis and anaerobic glycolysis, which inhibit the sodium channel activity (Lang et al., 1998; Foskett, 2000; Smets et al., 2002).

We investigated whether hypertonic mannitol has a beneficial effect in conditions of hypoxia by counteracting the effects induced by cellular swelling, i.e. extracting water osmotically out of the cells. The major renal actions of mannitol have already been mentioned in the previous chapter. It has been demonstrated that mannitol is able to minimize cell swelling due to metabolic inhibition (Powell et al., 1976).

In order to determine the presence of obstruction in the tubules and to assess whether this obstruction was the result of cellular swelling, intratubular pressure was measured in the proximal tubules before, during and after hypoxic stress in the kidney and in the presence and absence of hypertonic mannitol.
One of the problems with micropuncturing the kidneys is the prevention of excessive movement of the animal, which requires an intensive anaesthesia. Consequently it is difficult to control overall renal function influenced by systemic reactions, which might be detrimental for the kidney (Andreucci et al., 1978). The isolated kidney preparation has the advantage that it is fairly well fixed and is not exposed to systemic alterations of the circulation (Nizet, 1975; Maack, 1980; Schurek, 1980).

The measurement of the intratubular hydrostatic pressure was confined in this study to the superficial proximal tubules. This intratubular hydrostatic pressure opposes the intraglomerular hydrostatic pressure in the formation of the ultrafiltrate. In this set-up, only the so-called "free-flow" pressure was measured. This means that proximal tubular outflow to the distal tubules was not prevented. When an oil drop is introduced in the punctured tubule, the outflow to distal parts can be blocked and the resulting pressure is then called "stop-flow" pressure. "Stop-flow" pressure is frequently used to measure the activity of the tubuloglomerular feedback (TGF). Concomitantly the accompanying distal tubule is also punctured and retrograde flow to the macula densa is established. In this way stimulation of the macula densa can be controlled and the (re)activity of the tubuloglomerular feedback can be measured. An increase in GFR results in an increase of the stop flow pressure and the opposite is true for a decrease in GFR (Schnermann, 1998; Schnermann, 1999).
However, an increase in free flow pressure does not necessarily implicate an increase in GFR. It can also be the result of a decrease in proximal tubular outflow, which may be the consequence of distally located obstruction. This means that measurement of the free flow pressure must always be accompanied by assessment of the GFR, or distal delivery of tubular fluid. With these considerations, micropuncture studies were carried out on the isolated rabbit kidney perfused with autologous blood.

8.2 Methods and protocols

Intratubular hydrostatic pressure was measured in proximal tubules. For this purpose, sharpened microelectrodes with a tip diameter of 2 – 5 µm were used. These electrodes were filled with a 3 M KCl solution. The principle of these measurements has already been explained in the chapter on materials and methods.

Kidney surface was visualized with a Leica stereomicroscope. Proximal tubules were selected on sight (larger lumen, larger cellular layer, extensive peritubular capillary network compared with distal tubuli) and were randomly punctured. When visibility was limited, potential measured at the pipette tip was used to determine location. Lissamine green (0.2 – 1.0 g/100 ml) was detrimental for renal function and its use was discontinued.

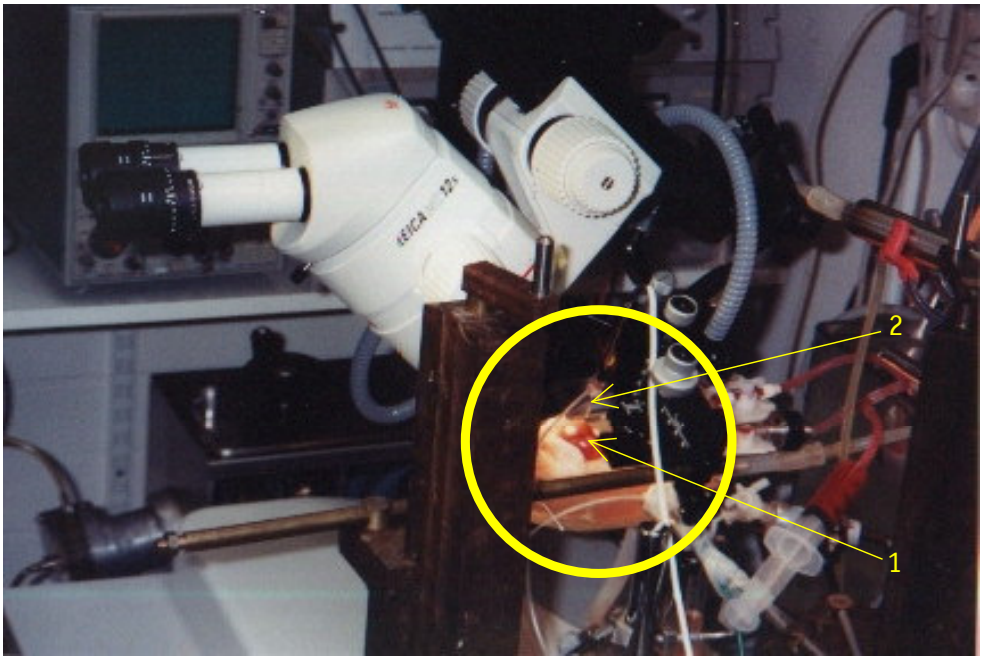

Figure 8.1: Micropuncture setup of the isolated rabbit kidney. In the encircled area the kidney (1, red) is seen with a micropipette (2, gray/white) penetrating the capsule and entering a proximal tubule.

Figure 8.1 is a photograph of the set-up. Figure 8.2 shows a drawing of the kidney surface as visualized by microscopic magnification. In general the techniques described by Andreucci and co-workers were used (Andreucci et al., 1978).

Urine collection started after the recovery period of 30 minutes, and was carried out in four periods (P1 – P4) of 30 minutes each. During each urine collection period, hydrostatic pressure was measured in at least four puncture sites. Each series contained five experiments.

Micropuncture data of P3 and P4 were pooled. In the first series, hypoxic stress was applied to the kidney during 25 - 30 minutes in P2, just by replacing the oxygen in the oxygenator with N_2 (96% N_2, 4% CO_2). In the second series of experiments, 15% mannitol was infused at a rate of 0.1 ml/min during P2 – P4, while the hypoxia was applied in P2. In the third series of experiments the effects of 15% mannitol infusion at 0.1 ml/min during P2 – P4 were investigated.

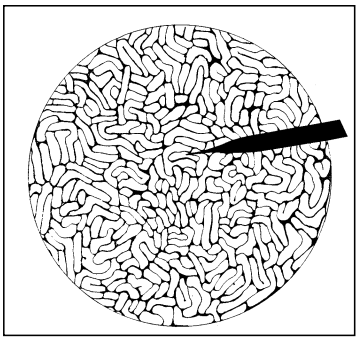

Figure 8.2: Schematic appearance of kidney surface as seen under microscopic visualisation. Modified from: Andreucci and co-workers (Andreucci et al., 1978).

8.3 Results

Relevant results are presented in table 8.1 and the course of the hydrostatic pressure in the proximal tubules of the perfused kidneys is presented in figure 8.3.

Exposing the kidney to hypoxia increases renal blood flow to a value more than two times higher than the basal value. After reoxygenation RBF decreases to the basal value. Infusing mannitol during the hypoxia period does not prevent the increase in flow. Mannitol alone increases the blood flow as well, but to a far lesser extent than the hypoxia.

There is no urine production during the application of hypoxia alone, making it difficult to estimate GFR if any should be present. Cessation of hypoxia leads to a partial recovery of the GFR, although it remains low (11% of control, 60 minutes after the hypoxia). When mannitol is infused, urine flow continues during hypoxia and GFR recovers better after

reoxygenation is installed. As has already been presented in the previous chapter, in a normal oxygen environment mannitol does not affect GFR significantly.

Table 8.1: Relevant results.

	Parameter	P1	P2	P3	P4
Hypoxia (n = 5)	RBF (ml/gkw.min)	2.2±0.4	5.5±0.4*	3.1±0.4*	3.3±0.4*
	GFR (µl/gkw.min)	378±34	-	24±37*	41±34*
	Uv (µl/min.g kw)	4.2±1.2	-	2.0±4.7	8.8±6.1
	Plasma K^+ (mEq/l)	3.1±0.4	4.7±0.4*	7.2±0.4*	4.2±0.4*
	K^+ excretion (mEq/gkw.min)	0.47±0.04	-	0.04±0.04*	0.25±0.04*
Mannitol & Hypoxia (n = 5)	RBF (ml/gkw.min)	2.0±0.4	5.6±0.4*	1.9±0.4	2.8±0.4*
	GFR (µl/gkw.min)	328±34	92±34*	110±34*$	170±34*$
	Uv (µl/min.g kw)	13±1.2$	28±5.4*	33±4.7*$	56±6.1*$
	Plasma K^+ (mEq/l)	2.7±0.4	6.4±0.4*$	6.2±0.4*	1.9±0.4$
	K^+ excretion (mEq/gkw.min)	0.53±0.04	0.47±0.04	0.23±0.04*$	0.24±0.04*
Mannitol (n = 5)	RBF (ml/gkw.min)	2.5±0.3	3.0±0.3*$	3.2±0.4*	3.4±0.4*
	GFR (µl/gkw.min)	298±35	306±54	231±39$	203±42*$
	Uv (µl/min.g kw)	12±2$	26±2*	39±3*$	50±4*$
	Plasma K^+ (mEq/l)	2.8±0.3	2.2±0.2*$	1.8±0.2*$	1.7±0.3*$
	K^+ excretion (mEq/gkw.min)	0.51±0.07	0.43±0.07	0.27±0.03*$	0.27±0.04*

***p < 0.05 vs.P1, , $p < 0.05 vs. hypoxia.**

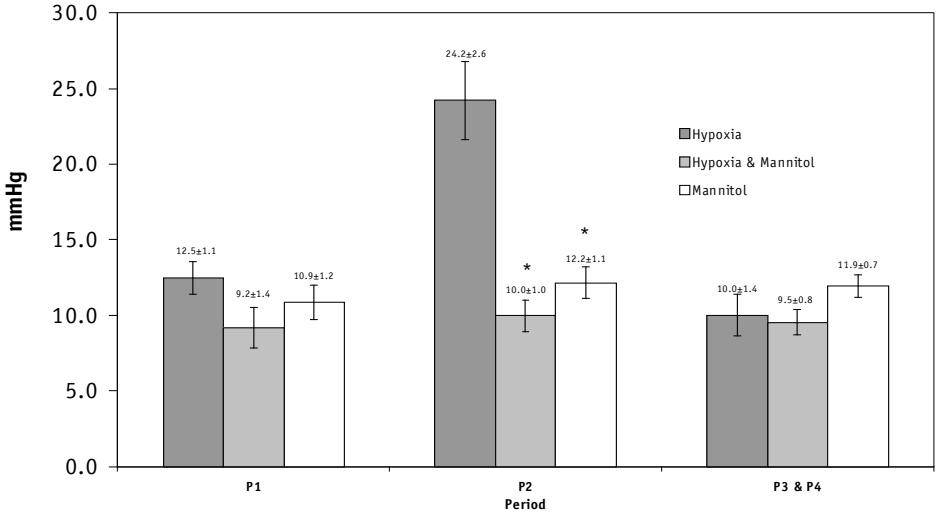

Figure 8.3: Course of the intratubular hydrostatic pressure in the proximal superficial part of the nephron. *p < 0.05 vs. Hypoxia.

Plasma [K^+] increases during hypoxia, but decreases again after oxygen supply has been reinstalled. The same pattern is seen in the presence of mannitol during and after hypoxia. Because of the lack of urine production, potassium excretion is zero during hypoxia. After the hypoxic stress excretion of potassium restarts, but remains low. In the presence of mannitol, potassium excretion follows identical patterns with and without hypoxic stress.
Intratubular hydrostatic pressure (figure 8.3) increased when hypoxia was applied. After reoxygenation, this pressure declined back to control values. Infusion of mannitol prevented the rise of tubular pressure during the hypoxia.

8.4 Discussion

With the huge rise (2 – 3 times control) of renal blood flow during hypoxia, one would have expected a high filtration rate, but on the contrary, urine production ceased (Dworkin and Brenner, 2000). The corresponding intratubular pressure rose in parallel with the RBF during P2. Therefore it is concluded that the cessation of urine production most probably is a consequence of tubular obstruction. This obstruction could be reversed by hypertonic mannitol infusion since it was observed that urine production did not entirely cease during hypoxia in the presence of hypertonic mannitol. However, the urine production was lower than the value measured in conditions with hypertonic mannitol infusion. No increase in intratubular pressure was observed during hypoxia in the presence of mannitol, despite the similar increase in RBF. Leyssac and co-workers measured a similar intratubular pressure under control conditions in rat kidneys in vivo (13.7 mmHg), but observed a significant increase of this pressure with infusion of a hypertonic mannitol solution (34.6 mmHg) (Leyssac et al., 2000). Another study with isolated rat kidneys, demonstrated no increase in intratubular pressure with mannitol infusion. However, the initial pressure (34 mmHg) was much higher than those observed in the present study and kidneys were perfused at the unphysiological temperature of 8°C (Lindstrom et al., 1999). The lower temperature was used in order to minimize the damaging effects of perfusion with an artificial perfusate (Alcorn et al., 1981; Lieberthal et al., 1987). However, during perfusion without mannitol they still observed tubular swelling, while histology was preserved with perfusion of hypertonic mannitol. The tubular swelling might still be the consequence of low oxygen content, supporting our findings here that mannitol improves GFR during hypoxia and that the low GFR can partly be explained by cellular swelling. In addition, the high proximal tubular pressure they measure initially can be explained by metabolic insufficiency, since

they observe a tendency for this pressure to decrease when perfusing with mannitol (31 mmHg).

It is also remarkable that mannitol improves GFR after hypoxia in P3 and P4 to a level that is close to the normal value. The improvement of GFR in P3 and P4 cannot be explained by a reduction of cell swelling, since oxygenation had been reinstalled and consequently swelling should have vanished, however the exact mechanism remains to be determined. In a study performed in rabbits, Hanley and Davidson demonstrated improvement of ischemic renal failure when the rabbits were pre-treated with mannitol (Hanley and Davidson, 1981). Even the use of isotonic mannitol seemed beneficial on renal function in a study performed on dogs and it is suggested that this might be due to a better maintenance of transglomerular hydrostatic pressure and secondary prevention of tubular swelling (Burke et al., 1983).
Lieberthal and co-workers demonstrated a beneficial effect of mannitol on ischemic renal failure in vitro as well as in vivo (Lieberthal et al., 1990).

Generally it has been proposed that obstruction in nephrons can be the consequence of renal cell cast forming or cell swelling (Lieberthal, 1997; Brady et al., 2000). In the present preparation obstruction could also be the result of the increased blood flow, with compression of the tubules. However it seems unlikely that the reduction of GFR is merely due to an obstruction of the tubular lumen following extratubular compression and leading to counter pressure, for after recovery of the RBF, following cessation of the hypoxia, GFR remains low. In addition, during hypoxia with mannitol there still is an increase in RBF, while intratubular pressure does not increase. This argues against the increased RBF as cause of major obstruction.

In the present preparation, the contribution of cast formation to the obstruction seems unlikely. Indeed the obstruction would be mechanical and still be present after cessation of the hypoxia and would probably not be removed by mannitol, since a transient increase in pressure was never observed during mannitol infusion.
Hence cellular swelling seems to be the most plausible cause to explain the obstruction.
Indeed, an increase in plasma potassium is observed during the hypoxic stress in P2 (4.7±0.4 mEq/L) and during normoxia in P3 (7.2±0.4 mEq/L). In fact the maximal rise in potassium was observed at the end of P2, and decreased thereafter, but, as explained

previously in the chapter on material and methods, the mean plasma potassium value per period was derived from the average of the values at the beginning and end of each period. This high value indicates cellular loss of potassium. The high potassium concentration cannot be the consequence of an imbalance of renal excretion and continuous infusion of K^+, since the latter was stopped as soon as the urine production had ceased.

After cessation of the hypoxia, plasma potassium decreases in P4, even when the infusion of K^+ (0.30 mEq/gkw.min average) remains higher than the K^+ excretion. This indicates that the potassium is again taken up by the cells via the restored activity of the Na-K-ATPase. In the presence of hypertonic mannitol, the decrease in plasma potassium is more dramatic, even when K^+ excretion is lower than the infused amount (0.36 mEq/gkw.min average) during P3 and P4. This indicates that the hypertonic mannitol shrinks the cells and that regulatory volume increase (RVI) results in extra accumulation of potassium in the intracellular compartment. A similar situation is observed during infusion of hypertonic mannitol without hypoxia (K^+ infusion = 0.32 mEq/gkw.min).

In addition, the role of cell swelling under hypoxic conditions has also been considered in other studies (Powell et al., 1976; Zager et al., 1985; Mason et al., 1989; Smets et al., 2002).

In summary the present results indicate that:

1. Hypoxic stress leads to cellular swelling
2. Cellular swelling contributes to an obstruction of tubular lumen, leading to an increase in tubular pressure and a decrease in GFR
3. Hypertonic mannitol reduces cellular swelling and eventually leads to improvement and even recovery of renal function during and after hypoxic stress.
4. The present preparation is suitable for micropuncture studies.

The use of mannitol to prevent acute renal failure in the clinic has been recommended by some investigators (Better et al., 1997; Sirivella et al., 2000), whereas cases of renal failure after mannitol infusion have also been reported (van Hengel et al., 1997; Matsumura, 2001). It is clear that there is no consensus about the treatment of acute renal failure with mannitol infusion. Further investigation is required.

Summary and general conclusion

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The kidneys perform an immense task in regulating extracellular fluid volume and osmolality. We have developed and characterized an isolated rabbit kidney preparation that is perfused with autologous blood. Isolated preparations have the advantage over animal studies in that research can be performed independent of systemic influences that affect the whole body. Moreover these preparations allow micropuncture studies in controlled conditions, apart from large deviations of perfusion pressure or movements of the animal. In addition, pharmacological agents can be supplied in very high doses, without affecting systemic functions that may influence renal function.

In chapter 1 a comparison was made with other available preparations and methods. Table 1.7 summarizes the findings. Perfusion of isolated kidney preparations can be accomplished by various kinds of perfusate. We chose to use autologous blood in order to mimic renal function optimally. With the use of blood the kidney is able to concentrate or dilute the urine produced, a phenomenon that has not been observed using an artificial perfusate. Moreover, there is less decrease of renal integrity during perfusion with blood, oxygen supply remains maximal, even in the more susceptible regions of the kidney. There is evidence available, that the presence of red blood cells in the perfusate prevents damage to the thick ascending limb of Henle's loop (Alcorn et al., 1981; Lieberthal et al., 1987).
There are also some disadvantages. The use of blood often leads to an intense vasoconstriction of the perfused kidney and discourages its application. The low blood flow during vasoconstriction prevents the kidney to perform in a normal fashion.

A gentle handling of the kidney and its autologous blood is required, in order to ensure proper function and rational blood flow. The complete methodology is described in chapter 3. In short, the following precautions should be respected in order to acquire optimal function of the preparation:

- During surgery the kidney should be touched minimally and in a very gentle fashion.
- Extensive bleeding during surgery should be stopped immediately.
- Perirenal fat should be removed as much as possible, since this appeared to be a major source of blood loss during perfusion.

- The perfusion should start within 3 minutes after blood is withdrawn from the donor animal.
- Ischemic time between catheterisation and connection to the perfusion apparatus should not exceed 6 minutes.
- Mechanical stirring of the blood should be avoided.
- Blood volume ideally should not exceed 3 times the kidney weight.
- Blood replacement during perfusion has to be done as slowly as possible.

The kidneys were perfused with autologous heparinised blood. Blood was oxygenated with a gas mixture of 4% CO_2 and 96% O_2, saturated with water.

Chapter 4 dealt with the normal function of this isolated perfused rabbit kidney. Renal blood flow (2 – 3 ml/min.g kw) and GFR (200 – 300 µL/min.g kw) were comparable with reports on in vivo studies performed on rabbits (Kozma et al., 1974; Sejersted, 1977; Wong et al., 1986). The preparation demonstrated partial autoregulation of renal blood flow with varying perfusion pressure. Glomerular filtration rate was autoregulated more completely. Autoregulation of renal perfusate flow and glomerular filtration rate is mostly absent in preparations perfused with artificial solutions. Fractional sodium excretion was higher than the values measured in vivo, although there are a few reports available in which a higher fractional excretion of sodium is reported both in vivo and in vitro (Wong et al., 1986; Arnaud et al., 2000).

An almost linear relationship was observed between perfusion pressure and sodium/water excretion. Thus this preparation demonstrated pressure diuresis and natriuresis.
Glucose and phosphate excretion showed a filtered treshold load. In the physiological ranges of plasma [glucose], little excretion was observed, indicating that proximal tubules of these kidneys functioned normally.

Potassium excretion was somewhat higher than data from the literature. The rabbits used in this study manifested a rather high transtubular potassium gradient (TTKG) indicating a high mineralocorticoid activity. Throughout experiments the TTKG remained high, with a gradual decrease towards the end. This high TTKG might explain the rather high potassium excretion.

The perfused kidneys were able to dilute and concentrate urine, depending on the absence or presence of arginine vasopressin (AVP). A urine osmolality of > 600mOsm/kg was obtained throughout all the experiments performed in the presence of AVP. Absence of AVP in the perfusate led to a diluted urine (233 – 275 mOsm/kg). The presence of urea in the perfusate was necessary in order to achieve maximal urine osmolality. Omission of urea from the compensating infusion gradually decreased urine osmolality.

The effects of adenosine on the function of this preparation were investigated in chapter 5. Adenosine increased renal blood flow and decreased GFR, indicating vasodilatation of the efferent arteriole rather than the afferent arteriole. Renal concentrating capacity was compromised with adenosine. Both adenosine receptor A_1 and A_2 blockers did not change renal blood flow and GFR significantly. A_1 receptor blockade led to diuresis, while A_2 receptor antagonism revealed a natriuretic effect. These observations are generally in accordance with available data in the literature and suggest that adenosine indeed might be the mediator of the tubuloglomerular feedback (TGF) in the rabbit.

In chapter 6 effects of nitric oxide were investigated. Nitric oxide production inducer and precursor L-arginine increased renal blood flow while there was only a tendency to increase GFR. The observations suggest a possible enhancement of sodium reabsorption as well. Blockade of nitric oxide synthesis (L-NAME) led to a decrease of renal blood flow, GFR and Na^+ excretion. Urine osmolality was not affected by L-arginine or L-NAME.

Chapter 7 dealt with the effects of the diuretics, mannitol and furosemide, on this preparation. Mannitol increased the renal blood flow slightly but did not affect the GFR. Furosemide on the contrary had little effect initially on the RBF, but decreased the GFR significantly in a low dose. In both conditions there was a significant increase of sodium and water excretion. Furosemide had a strong kaliuretic effect, whereas mannitol did not increase the potassium excretion significantly. Renal concentrating capacity was not affected in a major way by mannitol, whereas furosemide compromised this capacity completely.

In chapter 8 hypoxic stress was applied to the preparation. The hypoxic stress led to a tremendous increase of RBF, whereas urine production ceased almost immediately. After

cessation of the hypoxic stress, GFR recovered slightly. These results suggested the presence of tubular obstruction. Hydrostatic pressure measured through micropuncture in proximal tubuli supported this hypothesis, since intratubular pressure increased during hypoxic stress and returned to the normal value after reoxygenation. It was assumed that the obstruction might be due to cell swelling. Hypertonic mannitol was infused during the hypoxic stress. The results observed then, supported the assumption of cell swelling, since intratubular pressure did not increase and urine production during hypoxia did not cease completely. In addition GFR recovery was better after the hypoxic stress. The beneficial effect of mannitol on acute renal failure is still under discussion, since there are also reports on decline of renal function when hypertonic mannitol was infused, however in the present preparation its effect is beneficial.

In conclusion it can be said that, contrary to what is presented in the majority of the literature, it is possible to perfuse an isolated rabbit kidney with (autologous) blood and obtain a preparation that functions relatively well. The use of blood is preferred above artificial solutions, because with the former the kidney is still able to concentrate or dilute urine and the function of susceptible parts of the nephron is not compromised.
The investigation of ischemic renal failure as well as therapeutic implications of pharmacological agents used in this preparation could improve our understanding of the complex pathophysiological mechanisms of acute renal failure.

Samenvatting en algemene conclusie

Samenvatting en algemene conclusie

De nieren hebben een heel belangrijke taak in het regelen van het volume en de osmolaliteit van de extracellulaire vloeistof. In ons laboratorium hebben wij een geïsoleerde konijnennier, die met autoloog bloed wordt geperfundeerd, op punt gezet en gekarakteriseerd. Geïsoleerde preparaten hebben het voordeel ten opzichte van dierstudies in het feit dat zij onafhankelijk van systemische invloeden onderzocht kunnen worden. Bovendien kunnen micropunctiestudies op deze preparaten uitgevoerd worden in een situatie waarbij grote variaties in perfusiedruk en bewegingen van het dier niet voorkomen. Daarnaast kunnen farmacologische preparaten in een heel hoge dosering worden toegediend, zonder dat er systemische veranderingen optreden, die de nierfunctie zouden kunnen beïnvloeden.

In hoofdstuk 1 worden verschillende geïsoleerde preparaten en methoden vergeleken met elkaar. In tabel 1.7 worden de bevindingen samengevat. Perfusie van geïsoleerde nieren kan plaatsvinden met behulp van verschillende soorten perfusievloeistoffen. In onze situatie werd autoloog bloed gebruikt, om de natuurlijke situatie zo goed mogelijk na te bootsen. Bovendien is de geïsoleerde nier daardoor in staat om de geproduceerde urine te concentreren, iets wat niet geobserveerd wordt bij preparaten waar men een artificiële perfusievloeistof gebruikt. Gedurende perfusie met bloed is er ook minder achteruitgang van de nierfunctie. De beschikbaarheid van zuurstof blijft maximaal, ook in de gebieden waar er normaal reeds een lage zuurstofspanning heerst. In het verleden is er aangetoond dat de aanwezigheid van rode bloedcellen in de perfusievloeistof beschadiging aan de dikke opstijgende tak van de lis van Henle kon voorkomen (Alcorn et al., 1981; Lieberthal et al., 1987).
Het gebruik van bloed heeft ook enkele nadelen. Zo kan er een intensieve vasoconstrictie optreden. De lage doorbloeding in dit geval leidt tot het slecht functioneren van de nier.

De methode wordt uitgebreid beschreven in hoofdstuk 3. Voorzichtig omgaan met de nier en het bloed dat gebruikt wordt voor de perfusie is in elk geval noodzakelijk. In het kort moeten de volgende punten in acht worden genomen, wil men een optimale functie van de geïsoleerde nier verkrijgen:

- Het aanraken van de nieren moet tot een minimum beperkt worden en dient heel voorzichtig te geschieden tijdens de operatie.

- Overmatig bloedverlies tijdens de operatie dient onmiddellijk aangepakt te worden.
- Perirenaal vet dient maximaal verwijderd te worden, aangezien dit vaak een bron van bloedverlies blijkt te zijn tijdens perfusie.
- Maximaal 3 minuten nadat het bloed uit het dier gehaald wordt, dient de perfusie gestart te worden.
- De ischemietijd tussen catheterisatie en aansluiting op de perfusie-apparatuur dient niet langer dan 6 minuten te bedragen.
- Overmatig schudden van het bloed moet voorkomen worden.
- Het circulerend volume aan bloed mag in de ideale toestand niet meer dan drie maal het gewicht van de nier bedragen.
- Vervanging van het bloed dient heel langzaam en voorzichtig te geschieden.

De nieren werden geperfundeerd met geheparinieerd autoloog bloed. Oxygenatie van het bloed vond plaats middels een gasmengsel dat 4% CO_2 en 96% O_2 bevatte en verzadigd was met water.

In hoofdstuk 4 wordt de normale functie van deze geïsoleerde nier van het konijn behandeld. De renale doorbloeding (2 – 3 mL/min.g kw) en GFR (200 – 300 µL/min.g kw) kunnen vergeleken worden met studies die in vivo verricht zijn op konijnen (Kozma et al., 1974; Sejersted, 1977; Wong et al., 1986). Het preparaat vertoonde partiële autoregulatie van renale doorbloeding bij een variërende perfusiedruk. Autoregulatie van de GFR was beter. Preparaten die geperfundeerd worden met een artificiële perfusievloeistof vertonen over het algemeen nauwelijks autoregulatie van renale doorbloeding of GFR. De fractionele excretie van natrium leek hoger te zijn dan de waarden, die gemeten worden tijdens in vivo studies, hoewel er toch enkele publicaties zijn die een hogere waarde rapporteren zowel in vitro als in vivo (Wong et al., 1986; Arnaud et al., 2000).

Er werd een lineair verband aangetoond tussen perfusiedruk enerzijds en de uitscheiding van water en natrium anderzijds. Daaruit kon geconcludeerd worden dat dit preparaat druk diurese en natriurese vertoont.

Glucose en fosfaat vertoonden een drempelconcentratie, waarbij excretie duidelijk werd. Bij fysiologische plasma concentraties van glucose, was er nauwelijks excretie waar te nemen, hetgeen aangeeft dat de proximale tubuli van dit preparaat perfect functioneerden.

De excretie van kalium in dit preparaat was enigszins hoger dan de gegevens die in de literatuur beschikbaar zijn. Echter vertoonden de konijnen die in onze proeven zijn gebruikt reeds een heel hoge transtubulaire kalium gradiënt (TTKG), hetgeen indicatief is voor een hoge mineralocorticoïde-activiteit. Tijdens de experimenten zelf bleef deze TTKG hoog. Dit kan de relatief hoge kaliumexcretie verklaren.

Afhankelijk van de aan- of afwezigheid van arginine vasopressine (AVP), waren de geperfundeerde nieren in staat de geproduceerde urine te concentreren of te verdunnen. In de aanwezigheid van AVP werd een gemiddelde urineosmolaliteit die groter was dan 600 mOsm/kg gemeten tijdens de experimenten. Afwezigheid van AVP in de perfusievloeistof leidde al snel tot een verdunde urine (233 – 275 mOsm/kg). De aanwezigheid van ureum in het perfusaat bleek noodzakelijk te zijn voor het bereiken van de maximale urine osmolaliteit. Het weglaten van ureum uit het compenserende infuus leidde tot een graduele daling van de urineosmolaliteit.

In hoofdstuk 5 werden de effecten van adenosine op dit preparaat onderzocht. Adenosine gaf een verhoging van de renale doorbloeding en een verlaging van de GFR, waardoor kon worden aangenomen dat eerder vasodilatatie van de efferente dan van de afferente arteriole werd uitgelokt. Het vermogen om de urine te concentreren werd onderdrukt door adenosine. Adenosine receptor A_1 en A_2 antagonisten veranderden de renale doorbloeding en GFR nauwelijks. A_1 receptor antagonisme leidde tot een verhoogde diurese, terwijl A_2 receptor antagonisme meer een natriuretisch effect vertoonde. Deze bevindingen zijn over het algemeen in overeenstemming met de beschikbare gegevens uit de literatuur en suggereren inderdaad een rol voor adenosine als mediator van de tubuloglomerulaire terugkoppeling (TGF) in het konijn.

In hoofdstuk 6 werd het effect van stikstofoxide (NO) nagegaan. NO productie werd geïnduceerd door L-arginine en veroorzaakte een verhoogde renale doorbloeding. De GFR vertoonde ook de neiging om te stijgen. Onze bevindingen doen vermoeden dat de verhoogde NO productie ook een verhoging van de zoutreabsorptie moest veroorzaken. Het afremmen van de NO productie middels L-NAME leidde tot een daling van renale doorbloeding, GFR en natriumexcretie. De urineosmolaliteit werd in geen van beide gevallen beïnvloed.

De invloed van de diuretica, mannitol en furosemide, op dit preparaat komt in hoofdstuk 7 aan de orde. Mannitol verhoogde de renale doorbloeding enigszins, maar beïnvloedde de GFR niet. Daarentegen verlaagde furosemide de GFR wel en tastte de doorbloeding helemaal niet aan. In beide gevallen werd er een significant hogere water- en zoutexcretie waargenomen. Furosemide vertoonde bovendien een sterk kaliuretisch effect, terwijl dat veel minder uitgesproken was met mannitol. Het concentrerend vermogen van de nieren werd weinig aangetast door mannitol, terwijl furosemide dat bijna volledig elimineerde.

In hoofdstuk 8 werd het preparaat blootgesteld aan hypoxie. De hypoxische prikkel leidde tot een enorme verhoging van de renale doorbloeding, terwijl de urineproductie vrijwel onmiddellijk ophield. Na de hypoxie, kon enig herstel van de GFR waargenomen worden. Deze resultaten suggereerden de aanwezigheid van tubulaire obstructie. Deze hypothese werd ondersteund door de waarneming van een verhoogde hydrostatische druk in de proximale tubuli tijdens hypoxie, terwijl deze druk naar een normale waarde terugkeerde na de hypoxie. De mogelijkheid van zwellen van de tubulaire cellen werd overwogen. Doordat de intratubulaire druk in de aanwezigheid van hypertoon mannitol niet steeg tijdens de hypoxie, kon redelijkerwijze worden aangenomen dat het mechanisme van cellulaire zwelling tijdens hypoxie ook bijdroeg aan de tubulaire obstructie. Bovendien stopte de urineproductie in de aanwezigheid van hypertoon mannitol niet tijdens de hypoxie en was het herstel van de GFR na de hypoxie ook beter dan in de onbehandelde groep.
Het voordelig effect van mannitol bij acute nierinsufficiëntie is nog steeds een punt van discussie, aangezien er publicaties zijn die gewag maken van een achteruitgang van de nierfunctie bij het toedienen hiervan. Hoe dan ook blijkt mannitol toch een gunstig effect te hebben in ons model.

Over het algemeen kan geconcludeerd worden dat, in tegenstelling tot wat er beweerd wordt in de literatuur, het wel mogelijk is om een geïsoleerde konijnennier te perfunderen met (autoloog) bloed en een relatief goed functionerend preparaat te verkrijgen. Het gebruik van bloed moet eerder geprefereerd worden, aangezien het gebruik van artificiële oplossingen leidt tot achteruitgang van het concentrerend vermogen van de nier en beschadiging van delen van het nefron, die gevoelig zijn voor zuurstofgebrek.

Het onderzoek van ischemisch nierfalen, alsook het toepassen van farmacologische stoffen op dit preparaat, kunnen ons inzicht omtrent de ingewikkelde pathofysiologische mechanismen van acute nierinsufficiënte bevorderen.

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Curriculum vitae

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Publication in International Journal

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Posters

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