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The Influence of the Adenosine A_1-receptor on Tubuloglomerular Feedback and Renin Release

BY

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Abstract

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The kidneys play a vital role in the maintenance of extracellular fluid and electrolyte balance and blood pressure. Adenosine, acting through the adenosine A_1-receptor (A_1R), and nitric oxide have been implicated in several of the regulatory mechanisms in the kidney. The A_1R has been found to be present in the renal vasculature, primarily in the afferent arterioles, and in the proximal tubules. The tubuloglomerular feedback mechanism (TGF) is an important regulator of renal vascular tone and glomerular filtration rate. The aim of these investigations was to further elucidate the role of adenosine, acting through the A_1R. Investigations on adenosine's renal effects were performed on transgenic mice lacking the A_1R.

TGF response, elicited by increased distal salt load, was completely abolished in the A1R knockout (A_1R -/-) mice. Basal plasma-renin levels were found to be ~2-fold higher in the A_1R -/- compared to the A_1R wild-type (A_1R+/+) mice. However, salt intake induced inverse changes in plasma-renin levels, indicating that adenosine tonically inhibits macula densa stimulated renin release. Anesthetized and conscious A_1R -/- mice, measured telemetrically, had an increased blood pressure, which could be due to the increased plasma-renin levels. Despite the high plasma-renin levels, increased urinary sodium excretion was also observed in the A_1R -/- animals. Ischemia caused a decrease in renal function in both A_1R+/+ and A_1R -/- mice. Ischemic preconditioning protected the A_1R+/+ mice from subsequent ischemic episode but had no protective effect on the A_1R -/- mice.

Acute extracellular volume expansion greatly attenuates TGF sensitivity, thus facilitating the elimination of excess fluid. Acute inhibition of nNOS in volume-expanded rats was found to re-establish the attenuated TGF response caused by acute extracellular volume expansion.

The results show that adenosine, acting through the A_1R, plays an important role in mediating TGF response and consequently, regulating renin release, blood pressure, electrolyte balance and other vital renal mechanisms.

Keywords: adenosine, tubuloglomerular feedback, renin release, blood pressure, micropuncture

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To my plickor, Lina & Isa

List of Papers

I. **Neuronal Nitric Oxide Synthase Inhibition Sensitizes the Tubuloglomerular Feedback Mechanism after Volume Expansion.**
Brown R, Ollerstam A, and Persson AEG
2004, Kidney Int. 2004 April Vol. 65, pp1349-1356.

II. **Abolished Tubuloglomerular Feedback and Increased Plasma Renin in Adenosine A_1-receptor Deficient Mice.**
Brown R, Ollerstam A, Skøtt O, Johansson B, Gebre-Medhin S, Fredholm B and Persson AEG. 2001, Am J Physiol Regulatory Integrative Comp Physiol, 281: R1362-R1367

III. **The Influence of the Adenosine A_1-receptor on Blood Pressure and Regulation of Renin Release.**
Brown R, Thorén P, Skøtt O, Fredholm B and Persson AEG,
2004, manuscript

IV. **Ischemic Preconditioning does not Protect against Renal Injury in Adenosine A_1-receptor Knockout Mice.**
Brown R, Larsson E, Fredholm B, and Persson AEG
2004, manuscript

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Abbreviations

ΔP_{SF}	Maximal TGF response
7-NI	7-Nitro indazole
A_1R	Adenosine A_1-receptor
A_1R-/-	Adenosine A_1-receptor knockout mice
A_1R+/-	Adenosine A_1-receptor heterozygous mice
A_1R+/+	Adenosine A_1-receptor wild-type mice
Ang II	Angiotensin II
GFR	Glomerular filtration rate
HS	High-salt
i. p.	Intraperitoneal
i. v.	Intravenous
JGA	Juxtaglomerular apparatus
L-NAME	N_ω-nitro-L-arginine methyl ester
LS	Low-salt
MAP	Mean arterial blood pressure
MD	Macula densa
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NOS	Nitric oxide synthase
NS	Normal-salt
P_{FF}	Proximal tubular free-flow pressure
PRC	Plasma-renin concentration
P_{SF}	Stop flow pressure
RBF	Renal blood flow
SD	Salt-deficient
SD	Salt-Deficient
SNGFR	Single-nephron glomerular filtration rate
TGF	Tubuloglomerular feedback
TP	Turning point
VE	Volume expansion

INTRODUCTION

Maintaining a relatively constant volume and stable composition of the body fluids is essential for homeostasis. The kidneys are considered to be the most important regulatory organ for controlling homeostasis, since they control not only the concentration of waste products from metabolism, but also the osmolality, volume, acid-base status ionic composition of the extracellular fluid, and indirectly regulate these same variables within the cells. This is achieved by regulating the concentrations of sodium, potassium, and hydrogen ions and excreting end-products of metabolism. Besides controlling homeostasis, the kidney also plays a central role in blood pressure regulation.

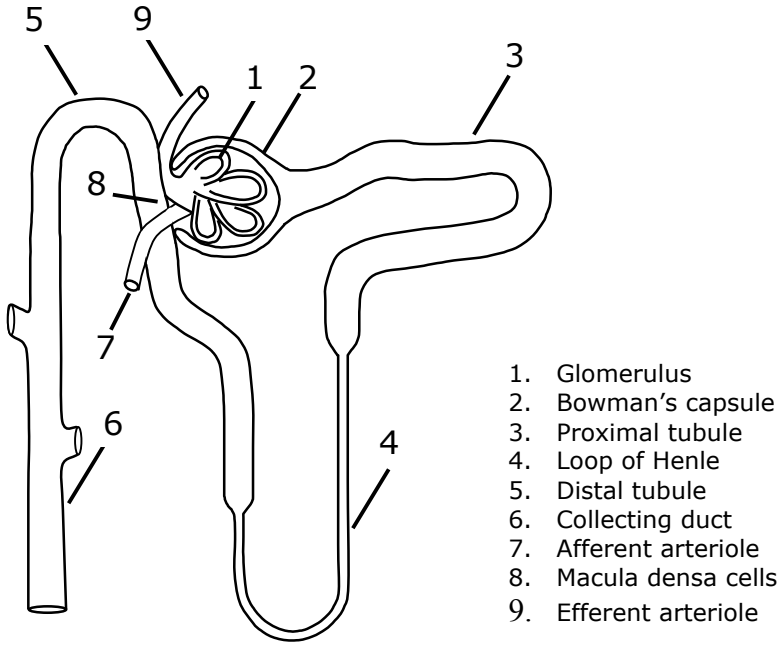

Figure 1. *Schemiatic drawing of the nephron.*

The human kidney consists of approximately one million nephrons, which are the functional units of the kidney (Fig. 1). Filtration, reabsorption and secretion takes place in each nephron. The nephron consists of a vascular and a tubular portion. The kidney receives approximately 20% of the cardiac output. After blood enters the kidneys through the renal arteries, the vessels divide several times until they reach the one million glomeruli through the afferent arterioles. In the glomerulus approximately 20% of the plasma is filtered off into the tubule system through Bowman's capsule. The blood then exits the glomerulus via the efferent arteriol, eventually leaving the kidney through the renal vein.

The filtered fluid, or primary urine, consists of almost protein-free plasma, and is collected in the tubular part of the nephron. The primary urine enters the proximal tubule and is transported through the loop of Henle and distal tubule where it is finally led through the collecting duct into the renal pelvis.

A fundamental feature of the nephron is that the distal tubule always returns to its own glomerulus, where it comes in contact with the afferent and efferent arterioles. This structure is known as the juxtaglomerular apparatus (JGA) and was first described by Golgi in 1898 and permits interaction between tubular epithelial cells and the smooth muscle cells of the afferent and efferent arterioles (Golgi, 1889). The JGA contains specialized structures in the walls of the afferent and efferent arterioles and of the distal tubule. The specialized cells in the distal tubule are modified tubular epithelial cells called macula densa (MD) cells and respond to the composition of the fluid within the tubule. The specialized granular cells found more predominantly in the afferent than efferent arteriole wall exhibit endocrine features and are the site for the production and release of the hormone renin.

The normal formation of urine is a fine balance between glomerular filtration rate (GFR) and tubular reabsorption of water and electrolytes. Since the tubules have a limited capacity for water and electrolyte transport, changes in GFR can result in severe and even life-threatening disturbances in the body's fluid homeostasis. On a standard western diet, only about 0.5-1% of the sodium filtered in the glomerulus of the kidney is excreted, whereas more than 99 % is reabsorbed along the tubular system of the kidney. This implies a fine coordination of glomerular filtration and subsequent reabsorption of fluid and electrolytes. A difference of as little as 5% would lead to a

daily net loss of about one-third of the total extracellular fluid volume, a situation that would inevitably lead to vascular collapse. Thus, kidney function is closely regulated by several intra- and extra renal mechanisms.

Autoregulation

One of the mechanisms to achieve a stable fluid balance is renal autoregulation. Renal autoregulation is the capability of the kidney to maintain constant renal blood flow (RBF) and GFR relatively constant over a wide range of perfusion pressures (Arendshorst et al., 1975; Baer and Navar, 1973) (Fig. 2). This ensures a relatively stable load of solutes to the tubular system allowing for precise regulation of reabsorption and secretion in the tubules. Two different mechanisms are responsible for the autoregulation of GFR and RBF, the myogenic response and the TGF system.

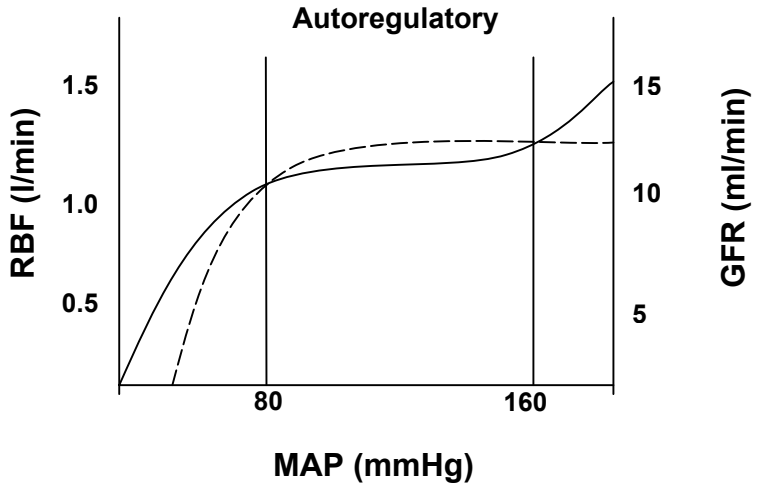

Figure 2. *Augoregulation of glomerular filtration rate (GFR ---) and renal blood flow (RBF —). GFR and RBF remain fairly stable during variatonf of mean arterial pressure (MAP) between 80 and 160 mmHg.*

Myogenic response

The myogenic response is an intrinsic property of the vasculature, which elicits automatic contraction of the smooth muscle fibers in the vessel wall in

response to increased perfusion pressure. The vasoconstriction reduces the transmural tension and flow and thereby decreases perfusion pressure.

Tubuloglomerular Feedback

The TGF mechanism is a negative feedback system, which couples ambient distal tubular flow to afferent arteriolar tone and thus, glomerular capillary pressure. Activation of the TGF elicits two responses; a change in GFR by altering afferent arteriole tone and an alteration in renin release from the granular cells. The TGF mechanism was first described in 1964 and plays an important role in the daily regulation of extra-cellular fluid volume and renin release. (Guyton et al., 1964; Thurau, 1964) The TGF depends on the special anatomical arrangement of the juxtaglomerular apparatus and was described by Golgi in 1889. It consists of the macula densa (MD) cells located in the initial portion of the distal tubule and juxtaglomerular cells in the walls of the afferent and efferent arterioles. The MD cells are a group of specialized epithelial cells in the distal tubule that can detect flow-dependant changes in luminal sodium chloride (NaCl) concentration. Ruyter first proposed a regulatory function of the MD cells in 1925 (Ruyter, 1925).

An increase in RBF or filtration pressure will result in an increased GFR and tubular flow rate. Because of the limited reabsorption capacity of the tubule, the increased flow rate will also cause an increase in the luminal concentration of NaCl at the MD site. When the MD cells detect increased distal flow and/or solute delivery they elicit a signal to the afferent arteriole. The signal causes a vasoconstriction of the afferent arteriole, resulting in a decreased perfusion pressure and GFR. Conversely, a decreased distal load causes perfusion pressure and GFR to rise.

The TGF can be divided into three steps, the sensing step in the MD cells, the signaling step from the MD cells to the afferent arteriole and the vasoconstrictor response of the afferent arterioles, resulting in decreased GFR. The sensing of the tubular fluid takes place by an increased influx of NaCl through the $Na^+/K^+/2Cl^-$ co-transporter located on the apical membrane (Gonzalez et al., 1998; Schlatter et al., 1989). It has been shown that loop diuretics can block the TGF mechanism (Odlind and Lonnerholm, 1982; Wright and Schnermann, 1974). The transmitted signal from the MD cells to the afferent arteriole is still rather unclear. A number of mediators and

modulators of the TGF signal have been proposed; ATP, angiotensin II, adenosin, arachidonic acid metabolites and nitric oxide (Braam and Koomans, 1995; Brown et al., 2001; Ichihara et al., 1998; Ito and Ren, 1993; Kurtz et al., 1998; Peti-Peterdi et al., 2003; Ren et al., 2000; Salomonsson et al., 1991; Thorup and Persson, 1994; Thorup and Persson, 1996; Wagner et al., 2000; Wilcox et al., 1992).

Under normal conditions the TGF exerts a suppressive effect on GFR (Briggs et al., 1984). The TGF response is not constant and the sensitivity and reactivity can be augmented or reset (Arendshorst, 1987). This resetting is an important function of the TGF. This enables the kidney to regulate fluid excretion depending on the prevailing condition. In a state of dehydration, when it is necessary for the body to prevent further fluid loss, the TGF sensitivity is increased even though the filtered load is lower than normal (Selen et al., 1983). Conversely, as seen in study I, volume expansion reduces TGF sensitivity, resulting in an increased urine production.

Nitric Oxide

The kidney is one of the many target organs for nitric oxide (NO). NO is derived together with L-citrulline from L-arginine. Renal physiology and pathophysiology involve a multitude of actions of NO ranging from the regulation of TGF, blood flow, renin secretion and glomerular filtration to glomerulonephritis and renal failure. Furchgott and Zawadzki first showed the physiological actions of NO in the vasculature in 1980 (Furchgott and Zawadzki, 1980).

NO is a short-lived molecule that can be synthesized by a group of three isoenzymes of nitric oxide synthases (NOSs) present in several tissues; the neuronal isoform, nNOS; the inducible isoform, iNOS; and the endothelial isoform, eNOS. nNOS and eNOS are reported to be constitutively expressed in the tissue and are Ca^{2+} dependent, while iNOS is inducible and Ca^{2+} independent. However, it has also been shown that iNOS is constitutively expressed in the kidney (Ahn et al., 1994; Mohaupt et al., 1994). nNOS is predominantly expressed in the MD cells in the kidney (Mundel et al., 1992; Thorup et al., 1993; Wilcox et al., 1992). nNOS has also been found to be expressed in the medullary thick ascending limb (McKee et al., 1994), inner

medullary collecting duct (Mattson and Bellehumeur, 1996; Roczniak et al., 1999; Roczniak et al., 1998; Wu et al., 1999) and in the principal cells of the cortical collecting duct (Bachmann et al., 1995; Wang et al., 1998).

NO plays an important role in vasomotor tone of the afferent arteriole (Baylis et al., 1990; Tolins et al., 1990), TGF (Thorup and Persson, 1996; Welch et al., 2000) and pressure natriuresis (Majid and Navar, 1997).

Adenosine

Adenosine is an endogenous nucleoside that modulates a number of physiological processes. In 1929, Drury and Szent-Györgi discovered the pronounced effects of adenosine on the cardiovascular system (Drury and Szent-Györgyi, 1929). Since then, many studies have been undertaken to study its effects in all the organs of the body. Adenosine plays important roles in normal metabolic processes and its concentrations are closely regulated.

Adenosine can be generated both intra- and extracellulary. Adenosine is synthesized intracellularly for the most part through two separate pathways, through the hydrolysis of AMP to adenosine by 5´-nucleotidase and through catabolism of S-adenosylhomocysteine (SAH) (Fig. 3). Extracellular adenosine might also be generated from cyclic AMP (cAMP), which could be released by tubular or vascular system (Mi and Jackson, 1995). Adenosine can either be released directly into the extracellular compartment or be generated from a precursor, ATP, ADP, AMP or cAMP through the action of ecto-5´-nucleotidases found on the surface of the cell membrane. Siragy and Linden found under normoxic conditions that the concentration of adenosine, measured from renal interstitial fluid was estimated to be approximately 63 nM in the cortex and 157 nM in the medulla (Siragy and Linden, 1996).

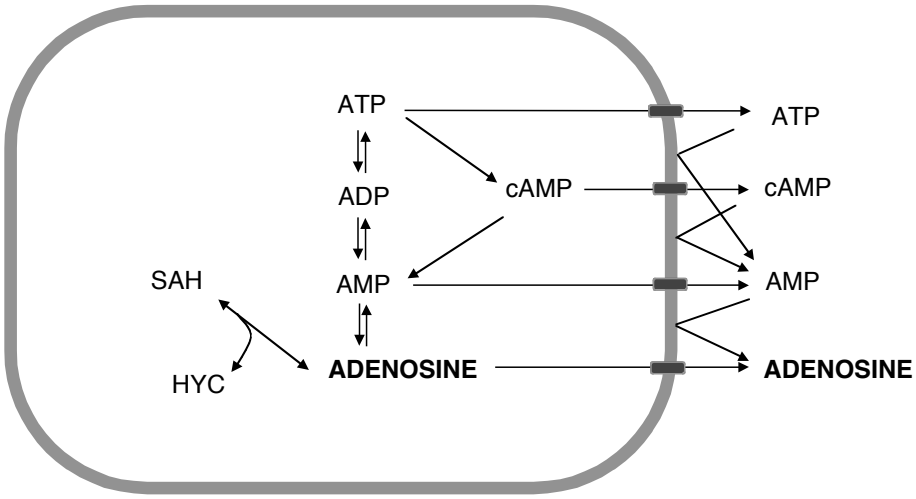

Figure 3. *Intra- and extracellular pathways for generation of adenosine. Adenosine is generated from the hydrolysis of ATP, from cyclic AMP (cAMP) from S-adenosylhomocysteine (SAH) together with homocystein (HYC) or extracellularly by ecto-nucleotidases.*

Adenosine is primarily regulated by renal metabolic activity and can be considered as a tissue hormone because of its short half-life, 1-3 seconds, in plasma (Shryock et al., 1990). After it is released it can interact with specific adenosine receptors. At present, four different G-protein coupled adenosine receptor subtypes have been characterized, namely, A_1, A_{2A}, A_{2B} and A_3 (Fredholm et al., 2001; Fredholm et al., 2000). The different subtypes have been shown to be expressed throughout the kidney, in the renal vasculature, juxtaglomerular apparatus,, glomeruli, tubules and collecting ducts (Spielman and Arend, 1991; Weaver and Reppert, 1992; Zou et al., 1999). The adenosine A_1-receptor (A_1R) and A_3-receptor are coupled to inhibitory G_I-proteins, which inhibit adenylyl cyclase, causing decreases of cAMP and through stimulating Phospholipase C and thereby increasing IP_3. The adenosine A_{2A} and A_{2B} receptors are coupled to stimulatory G_S-proteins, which stimulate adenylyl cyclase, causing an increase of cAMP. The A_1Rs have a greater affinity for adenosine analogues (in the nanomolar range), which is two or three fold higher than the A_2Rs (Olsson and Pearson, 1990).

In most of the vascular beds in the body the adenosine A_2R predominates and primarily causes vasodilation upon stimulation from adenosine. In the vasculature of the kidney, however, the A_1R predominates. Activation of the A_1R has the opposite effect compared to the A_2R and causes vasoconstriction. The A_1R are believed to be responsible for many of the renal actions of adenosine. In the kidney, which plays an important role in the regulation of body fluids and blood pressure, stimulation of the A_1R produces a constriction of the afferent arterioles (Osswald et al., 1980; Weihprecht et al., 1992). This vasoconstriction causes a reduction in GFR and renal blood flow and may have a have a key role in mediating the TGF response. In addition to its hemodynamic effects the A_1AR has been shown to be involved in regulating the release of renin (Skott and Baumbach, 1985), blood pressure (Guimaraes and Albino-Teixeira, 1996) and tubular NaCl reabsorption (Macala and Hayslett, 2002).

Renin-Angiotensin System

Tigerstedt was the first to describe the presence of renin in the kidney in 1898 (Tigerstedt and Bergman, 1898). Later, Goormaghtigh suggested that the granular cells in the wall of the afferent arteriole contained and secreted renin. Although the renin-angiotensin system (RAS) has many elements, the physiological functions are primarily exerted by angiotensin (ANG) II. ANG II is a potent vasoconstrictor and contributes to the maintenance of both short-term and long-term blood pressure regulation.

Renin is an enzyme that is synthesized and stored mainly within the granular cells in the JGA, and is not itself vasoactive. However, it acts on a plasma protein angiotensinogen, producing an inactive decapeptide, ANG I. As a result of the action of a converting enzyme largely present in lung epithelial cells, this substance loses two amino acids and becomes an active octapeptide, ANG II.

Besides it vasoconstrictive effects ANG II increases sodium reabsorption in the kidney, resulting in decreased salt and water excretion. This is achieved

by either directly affecting the tubular epithelial cells and by stimulating aldosterone release. ANG II has also been shown to increase the responsiveness of the TGF system (Huang et al., 1988; Mitchell and Navar, 1988; Schnermann, 1998).

AIMS OF THE INVESTIGATIONS

Study I Since volume expansion and increased nitric oxide production results in a decrease of tubuloglomerular feedback sensitivity, this study was designed to determine to what extent nitric oxide is involved in the effect of tubuloglomerular feedback sensitivity resetting that occurs upon extracellular volume expansion.

Study II The aim of this study was to investigate the effects that adenosine A_1-receptor deficiency had on the tubuloglomerular feedback response in newly developed adenosine A_1-receptor knockout mice. We also investigated adenosine's role in renin secretion.

Study III This study was designed to further elucidate the effects of adenosine A_1-receptor deficiency on blood pressure regulation and renin release in conscious mice treated with low-, normal- and high-salt diets.

Study IV This study was performed to examine the role of adenosine and the adenosine A_1-receptor in renal ischemic injury and to determine if the presence of the adenosine A_1-receptor is a necessary factory for ischemic preconditioning.

MATERIALS AND METHODS

Animals

The local animal ethics committee for Uppsala University approved all of the procedures for this study.

The experiments in study I were carried out on male Sprague-Dawley rats weighing 220-340g from Møllegaard Breeding center, Copenhagen, Denmark.

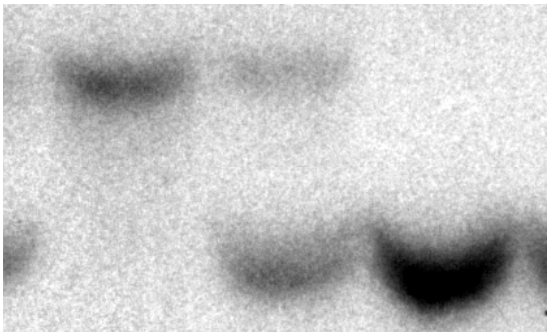

Figure 4. *Genotyping of the adenosine A_1-receptor knockout mouse. The autoradiogram of the Southern blots shows the three different mouse genotypes; the wild-type (+/+), heterozygous (+/-) and homozygous (-/-) knockout mouse.*

The mice in studies II, III and IV were carried out on female adenosine A_1-receptor deficient mice and their littermates weighing 20-35 g. These mice were developed by Professor Bertil Fredholm's group at the Department of

Physiology and Pharmacology, Karolinska Institute, Stockholm, Sweden (Johansson et al., 2001). The A_1R mice used in experiments were siblings from matings of A_1R +/- mice of a 50 % C57BL, 50 % 129/OlaHsd background. A_1R adenosine receptor knockout mice were genotyped with Southern blot analysis (fig 4). DNA from tail biopsies was digested with BamHI, run on an electrophoresis gel and probed with a BamHI-XhoI fragment derived from the immediate 5´ vicinity of the targeted exon. A wild-type allele generated a 20 kb fragment that hybridized, whereas in the targeted allele there was instead a 9 kb hybridizing fragment.

All animals had free access to food and water throughout the experimental periods.

Anesthesia

Rats

Rats were anesthetized by an intraperitoneal injection of Thiobutabarbital sodium (Inactin®, 120mg/kgBw, Sigma Chemical Co., St. Louis, MO, USA).

Mice

Anesthesia was induced by spontaneous inhalation of isoflorane (Forene®, Abbot Scandinavia AB, Kista, Sweden). The gas was a mixture of 40% oxygen and 60% nitrogen and contained approximately 2.2% isoflorane. The gas was delivered through a small animal vaporizer (Univentor 400 Anesthesia Unit, Univentor Ltd, Malta) to a breathing mask.

Surgical preparation (Studies I, II, III & IV)

After anesthesia was induced the animals were placed on a servo-regulated heating pad with a rectal probe and a body temperature of 37.5°C was maintained throughout the surgical procedure. Catheters were placed in the carotid artery and the jugular vein for blood pressure measurements and infusion of maintenance fluid (0.9% NaCl and 2% albumin, mice 0.35 ml/h; rats), respectively. The urinary bladder was catheterized for urine collection. In the studies where TGF, SNGFR, was measured (studies I, II & IV), a subcostal flank incision was made to expose the left kidney. The kidney was

dissected free from surrounding tissue, placed in a Lucite cup and fixed with a 3% agar-agar solution. The kidney surface was covered with paraffin oil to prevent drying throughout the experiments. Following an equilibration period of at least 45 minutes, TGF or SNGFR measurements were started. In study IV the left kidney and the renal artery and vein leading to the kidney were dissected free from surrounding connective tissue

Telemetric Blood Pressure Measurements (Study III)

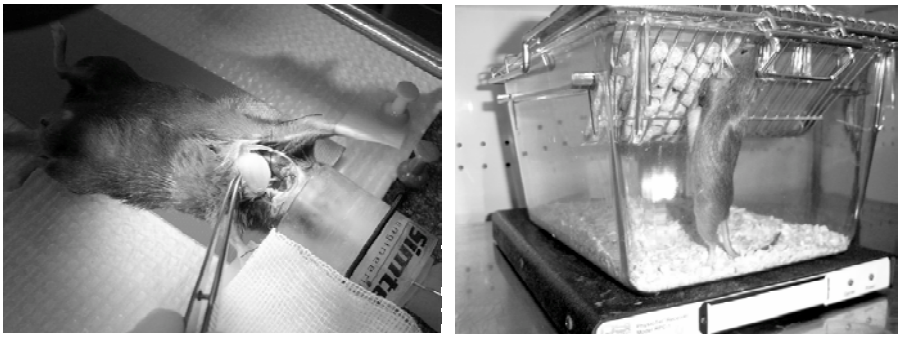

Figure 5. *Implantation of telemetric blood pressure device (left). Mouse implanted with telemetric blood pressure device housed in a cage that is placed on top of the receiver (right).*

Blood pressure was measured telemetrically with blood pressure transmitters (PA-C20; Data Science International, St. Paul, MN, USA). For implantation of the blood pressure transmitters the mice were anesthetized by spontaneous inhalation of isoflorane (Forene®) and placed on a servo-controlled heating pad to maintain body temperature at 37.5°C. A midline incision (~2 cm) was made from the lower mandible to approximately the sternum. The blood pressure catheter was inserted in the left carotid artery and advanced to the aortic arch. The transmitter body was placed subcutaneously along the right flank. The animals were allowed to recover at least 7 days before blood pressure recordings were commenced. The computer program PC-lab version 5.0 sampled calibrated values of blood pressure during the course of the experiment (Axenborg and Hirsch, 1993). Data was collected for five seconds every two minutes for one to five days at a time. Day (12 h) and night

(12 h) blood pressure readings were pooled and used for analysis. The recorded data was further analyzed using an Excel macro program.

Metabolic Cages (Study III)

Mice were individually placed in specially made metabolic cages for mice (Scientific Glass, Löberöd, Sweden) (Fig. 6). Food, either normal-salt (NS), high-salt (HS) or salt-deficient (SD) diet, and tap water were supplied ad libitum. 24-hours of acclimation was allowed before 24-hour measurements were performed. The average results for the last 24-hours were used for analysis.

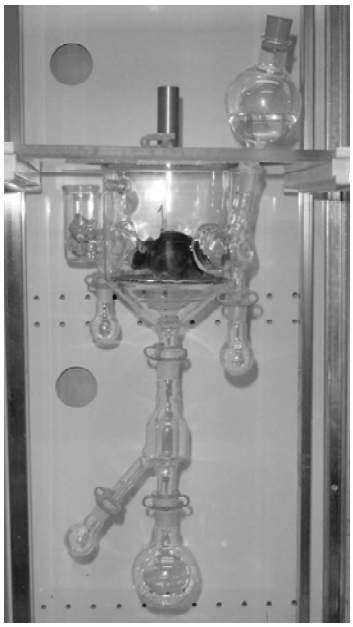

Figure 6. *Metabolic cage for mice.*

Whole Kidney Clearance Measurements (Studies II & IV)

Thirty minutes after the surgical procedures were completed, the mice were given a bolus infusion of 0.5 ΔCi [^{3}H]metoxy-inulin in 0.08 ml of normal

maintenance fluid into the jugular vein. 5 ΔCi/ml was then added to the maintenance fluid for continuous infusion. After a 45-minute equilibration period, 40-minute sampling periods were started. In study II mice were also given a 10mg/kg bolus of candesartan (AstraZeneca, Mölndahl, Sweden) and allowed to stabilize for 30 minutes, followed by a sampling period.

Total kidney urine flow-rate and sodium and potassium excretion were determined from urine samples taken through the catheter in the urinary bladder. At the midpoint of each collection period a blood sample was taken. The blood sample was centrifuged and aliquots of plasma and urine were analyzed in a multi-channel gamma counter (MR 300 Automatic Liquid Scintillation System, Kontron). Inulin clearance was then calculated as a measure of GFR according to:

$$GFR = [inulin_{urin}] \bullet V_U / [inulin_{plasma}]$$

where V_U denotes urine flow.

Urine Analysis (Studies II & III)

Urine volumes were determined gravimetrically. The urinary concentrations of sodium and potassium were assayed by flame photometry (FLM3, Radiometer, Copenhagen, Denmark). Osmolality of the urine was determined by depression of the freezing point (Model 3MO, Advanced Instruments Inc, Needham Heights, MA, USA).

Micropuncture

Stop-Flow Pressure Measurements (Studies I, II & IV)

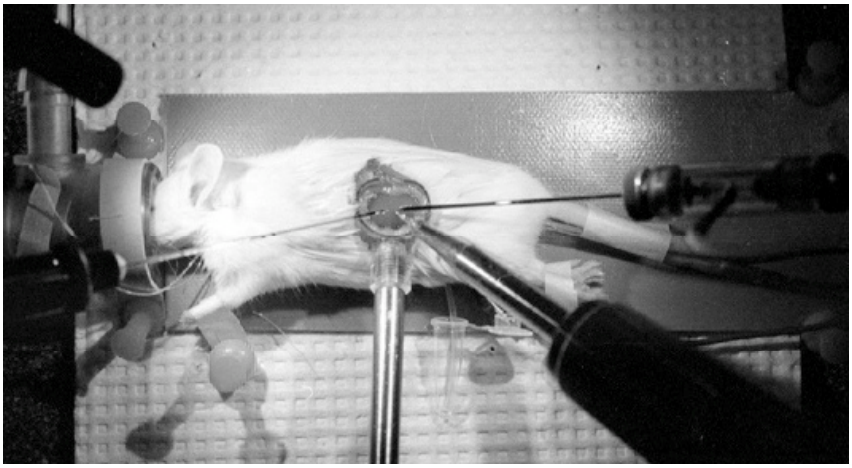

Figure 7*. Mouse prepared for renal micropuncture.*

TGF characteristics were determined by the stop-flow technique as shown in figure 7. Under a stereomicroscope, randomly chosen superficial proximal tubular segments were punctured with a sharpened glass pipette (outside diameter (O.D.) 3-5 Δm) filled with a 1 M NaCl solution stained with Lissamine green. The pipette was connected to a servo-nulling pressure system to determine the proximal tubular free-flow pressure (P_{FF}). By injection of the stained fluid, the tubular distribution of the kidney surface was determined. In nephrons where more than tree proximal segments were identified, a second pipette (O.D. 7-9 Δm) was inserted in the last accessible segment of the proximal tubule. This pipette was filled with an artificial ultrafiltrate solution (140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 4 mM $NaHCO_3$, 7 mM urea, 2 g/l Lissamine green, pH 7.4) and connected to a micro-perfusion pump.

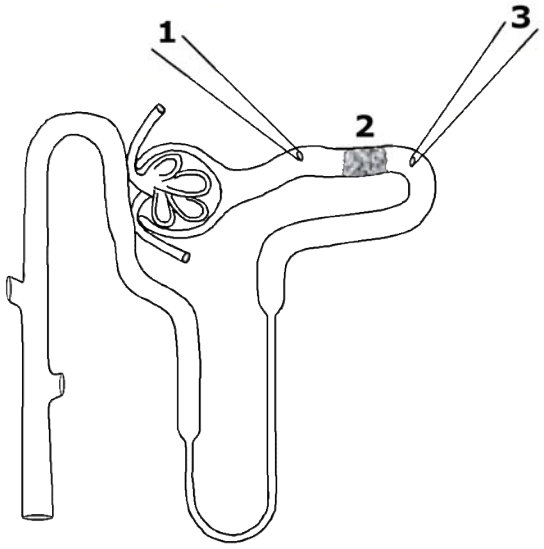

Figure 8. *Schematic drawing of the stop-flow pressure technique. A pressure pipette connected to a pressure measuring system (1). A wax block is placed downstream to the pressure pipette to stop the flow through the nephron (2). The loop of Henle is perfused with a perfusion pipette (3).*

Between these two pipettes a solid wax block was placed, with a third pipette (O.D. 7-9 Δm). To characterize the TGF signal, the pressure upstream to the block, the stop-flow pressure (P_{SF}), was measured at different perfusion rates (0-40 nl/min) in the loop of Henle. The perfusion rate was increased or decreased in steps of 2,5-5 nl/min and the maximal feedback response, $\Delta P_{SF,}$ was determined as the decrease in P_{SF} at maximal perfusion rate, compared with P_{SF} at zero perfusion. The tubular flow rate at which 50% of the maximal response was obtained, called the turning point (TP) was determined. By definition, the TP is a measure of TGF sensitivity.

For plotting the response curves in study I, a previously described normalization method was used (Selen et al., 1983). The normalized data were fitted by means of a nonlinear least squares curve-fitting program to the equation:

$$P_{SF} = P_{SF\,min} + \Delta P_{SF} / 1 + e^{w(PR - TP)}$$

where P_{SF} is the stop-flow pressure, ΔP_{SF}, the average decrease in stop-flow pressure and P_{SFmin}, the average minimum stop-flow pressure on increased distal delivery of fluid. TP is the turning point, PR is the end-proximal perfusion rate and w is the factor determining the width of the perfusion interval during which the stop-flow pressure responded.

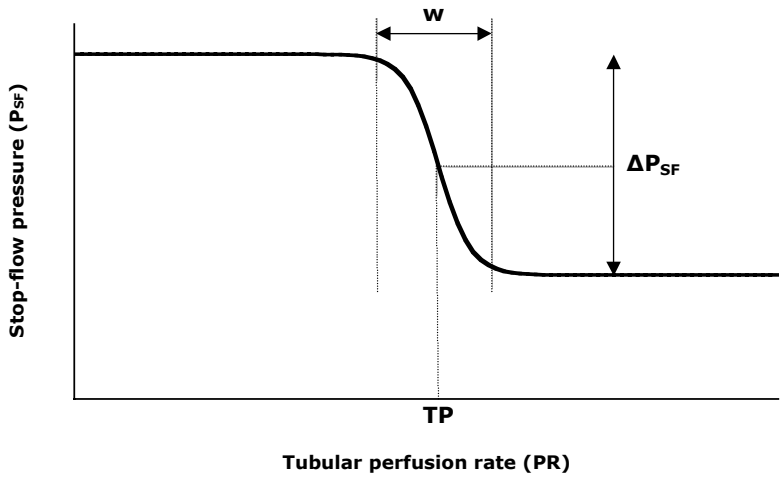

Figure 9. *The relationship between proximal tubular stop-flow pressure and the loop of Henle Perfusion rate.*

Single-nephron GFR Measurements (Studies I & IV)

For measuring SNGFR a 25ΔCi bolus dose of [^{3}H]metoxy-inulin was given i.v. followed by a continuous i.v. infusion of 50 ΔCi/h. Randomly chosen tubular segments on the surface of the kidney were punctured with a sharpened glass pipette (O.D. 7-9 Δm), filled with artificial tubular fluid (see above) stained with Lissamine green and connected to a micro-perfusion pump. The superficial tubular distribution of a nephron was visualized by injecting the stained fluid into a tubular segment. A collection pipette (O.D. 8-15 Δm) filled with castor oil stained with Sudan black was placed proximal to the perfusion pipette. The oil was injected into the tubule and tubular fluid was collected proximal to the oil block for three minutes, while the more distal tubular segment was perfused. The volumes of the collected samples were measured from the length in constant bore capillaries. Blood

samples were taken and SNGFR was determined from standard formulas (see section on whole kidney clearance measurements).

Sampling and Renin Assay (Studies II & III)

Immediately following anesthesia a blood sample was taken from the carotid artery, centrifuged and the plasma was frozen to -85°C. Plasma renin concentration was measured by radioimmunoassay of angiotensin I (ANG I) using the antibody-trapping technique (Lykkegard and Poulsen, 1976). 10 Δl of plasma from each sample was serially diluted between 50 and 1000 fold. Five microliters of each dilution were incubated in duplicates for 24 h together with rabbit ANG I antibody and renin substrate (about 1200 ng ANG I/ml) from 24 h nephrectomized rats and from which renin had been extracted by affinity chromatography. The reaction was stopped by addition of 1 ml cold barbital buffer, ANG I tracer was added and a radioimmunoassay was performed. Only results with linearity in serial dilutions were accepted. Renin values were standardized with renin standards obtained from the Institute for Medical Research (MRC, Holly Hill, London, UK), and are expressed in standard Goldblatt units (GU).

Ischemic Preconditioning (Study IV)

A subcostal flank incision was made to expose the left kidney. The left kidney and the renal artery and vein leading to the kidney were dissected free from surrounding connective tissue. Ischemia was induced by clamping the left renal vessels for a 45-minute time period. During this time the kidney was placed in it natural position with the abdomen closed so that the kidney temperature equaled body temperature. The animals were subjected to first, four cycles of 8-minute left renal ischemia separated by 5-minute reperfusion periods. Ischemia was then induced for a 45-minute period as described above.

Histological Preparations (Study IV)

Immediately after the end of the experiment the left and right kidneys were excised. They were then fixed in 4% buffered formalin, pH 7.3, and proc-

essed for routine histology. Four micrometer sections were used and stained with emtoxyline, Periodic Acid Shiff (PAS) and picro-Sirius for investigation of fibrosis.

Statistical Analysis (Studies I, II, III & IV)

All values are given as means ± SEM. Normally distributed parameters were tested for significance with the Student's paired or unpaired *t*-test. When multiple groups were compared, one-way analysis of variance (ANOVA) was employed, followed by the Bonferroni test for pairwise multiple comparisons. A *P*-value less than 0.05 was accepted for statistical significance.

Experimental protocols

Study I

The rats were divided into four groups following preparatory surgery for micropuncture.

- Normovolemic controls (N_C) - i.v. infusion of saline at 5 ml/h/kg.
- Volume expansion controls (VE_C) i.v. infusion of saline at 50 ml/h/kg.
- Volume expansion ($VE_{L\text{-}NAME}$) - i.v. infusion of saline at 50 ml/h/kg. The nonspecific NOS inhibitor, L-NAME was added to the artificial ultrafiltrate during micropuncture studies.
- Volume expansion ($VE_{7\text{-}NI}$) - i.v. infusion of saline at 50 ml/h/kg. a single i.p bolus dose of the nNOS specific inhibitor was given prior to micropuncture studies.

After volume expansion was commenced the animals were allowed to equilibrate for 90 mines prior to SNGFR and stop-flow micropuncture measurements. GFR, Na^+- and K^+-excretion measurements were made one after one hour of volume expansion and further 15-minutes subsequent to a single i.p. bolus dose of 7-NI (25mg/kg).

Study II

After surgical preparations $A_1R+/+$, $A_1R+/-$ and $A_1R-/-$ mice were allowed to equilibrate for 45-minutes before experiments were started. Stop-flow pressure was measured in the three genotypes while the loop of Henle perfusion rate was changed between 0 and 35 nl/min. GFR, Na^+ and K^+ excretion measurements were performed for 40 minutes. The mice were then given an i.v. bolus dose (10 mg/kg) of an angiotensin II AT_1-receptor blocker, and allowed to stabilize for 30-minutes, followed by a 40-minute sampling period. Immediately following anesthesia, a blood sample was taken from the carotid artery for determining plasma-renin concentration. Blood pressure was recorded throughout the experiments through a catheter in the carotid artery.

Study III

Adenosine $A_1R+/+$ and $A_1R-/-$ mice were divided into three different groups and given different standardized salt diets.

- Salt-deficient (0% NaCl)
- Normal-salt (0.7% NaCl)
- High-salt (7% NaCl)

The mice were allowed to equilibrate for 10 days on each diet prior to telemetric blood pressure, urinary excretion and plasma-renin measurements were performed.

Blood pressure measurements Telemetric blood pressure transmitters were implanted and the mice were allowed to recover for at least 7 days before blood pressure recordings commenced. Blood pressure was then continuously measured in the conscious mice for up to approximately one week. The salt diet was then changed and the mice were allowed to equilibrate for 10 days before new blood pressure recordings were started.

Urinary excretion measurements Mice were placed individually in metabolic cages for 24-hours to acclimatize to the cages before urinary excretion measurements were started. Urine was then collected after a 24-hour period. Average urine production, sodium and potassium excretion and urinary osmolarity was then determined for each mouse. Excretion measurements

were repeated for each mouse. The salt diet was then changed and the mice were allowed to equilibrate for 10 days on the new diet before new excretion measurements were started.

Plasma-renin measurements After at least 10 days on either the salt-deficient, normal-salt or high-salt diet a blood sample was taken to determine plasma-renin concentration.

Study IV

Adenosine A_1R+/+ and A_1R-/- mice were divided into three different groups.

- Control group mice were sham-operated
- Ischemia-reperfusion group - renal ischemia was induced by clamping the left renal vessels for 45-minutes.
- Ischemic-preconditioning group - the mice were first subjected to four cycles of 8-minute left renal ischemia separated by 5-minute reperfusion periods. Ischemia was then induced for 45-minutes.

After the ischemia surgical procedures the mice were allowed to recover for one week before kidney function was evaluated. Whole kidney GFR, SNGFR and free-flow measurements were then performed. After the completion of the functional studies both kidneys were removed and prepared for histological evaluation.

RESULTS AND COMMENTS

Study I

Neuronal Nitric Oxide Synthase Inhibition Sensitizes the Tubuloglomerular Feedback Mechanism after Volume Expansion

It is important for the body to be able to eliminate excess fluid and solute entering the body in order to maintain a constant milieu for the cells. During VE the TGF response is attenuated, which facilitates an increased fluid and solute excretion. This study was designed to investigate whether the inhibition of NOS or specifically nNOS re-establishes the attenuated TGF response caused by acute extracellular VE.

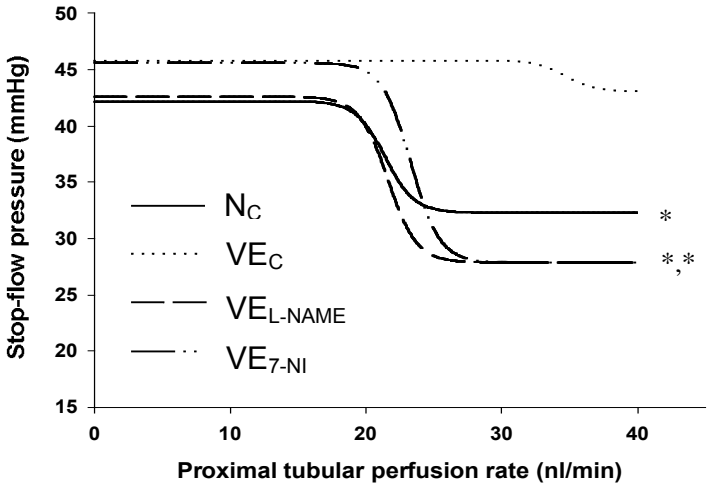

Figure 10. *Tubuloglomerular feedback response in normovolemic (N_C), 5% volume expanded (VE_C), 5% volume expanded with intra-tubular L-NAME ($VE_{L\text{-}NAME}$), and 5% volume expanded with 7-NI intraperitoneally ($VE_{7\text{-}NI}$) treated rats. Curves represent the proximal tubular stop-flow pressures after late proximal perfusion between 0 and 40 nl/min. * $P< 0.05$ vs. VE_C*

After the intra-tubular addition of the unspecific NOS blocker L-NAME (via the perfusion fluid), both TGF reactivity and sensitivity increased, as seen as an increased maximal ΔP_{SF} and leftward shift of the response curve as shown in figure 10. NOS inhibition also caused a reduction in SNGFR when distal perfusion was increased from 0 to 40 nl/min, indicating an increased TGF response. nNOS inhibition had a similar effect on SNGFR. nNOS inhibition with 7-NI also decreased whole kidney GFR following VE.

In conclusion, the main finding in this study is that the inhibition of nNOS, with the specific inhibitor 7-NI, sensitizes the greatly attenuated TGF response. The results presented in this study suggest that a functional nitric oxide system is important in mediating normal renal responses and that an increased production of or increased sensitivity to nitric oxide during VE plays an important role in the adaptive mechanisms of the TGF.

Study II

Abolished Tubuloglomerular Feedback and Increased Plasma Renin in Adenosine A_1-receptor-deficient mice

Adenosine and the adenosine A_1-receptor (A_1R) has earlier been implicated in the mediation of the TGF mechanism. Much of the earlier work that was performed relied on the use of pharmacological tools, which are not highly selective. A transgenic mouse strain lacking the A_1R was developed and because this deletion is very selective we were able to investigate its role in kidney function, specifically the TGF.

The A_1R+/+ mice were found to have a normal TGF response and P_{SF} decreased from 36.7 to 25.3 mmHg when distal perfusion rate was increased from 0 to 35 nl/min (Fig. 11a). The A_1R+/- mice showed similar changes in P_{SF} as the A_1R+/+ mice. In the A_1R-/-, however, this response was completely abolished as seen in figure 11b. The A_1R-/- mice were also shown to have increased blood pressure and plasma-renin concentration. There was no difference in GFR between the three genotypes, indicating no major changes in the glomerular filtration process. Despite an increased plasma-renin concentration in the A_1R-/- mice, when candesartan, an angiotensin II AT1-receptor blocker, was administered there were no changes in GFR. Sodium excretion was also elevated in the knockout mice, indicating not only hemodynamic effects of the A_1R, but also tubular effects in the kidney.

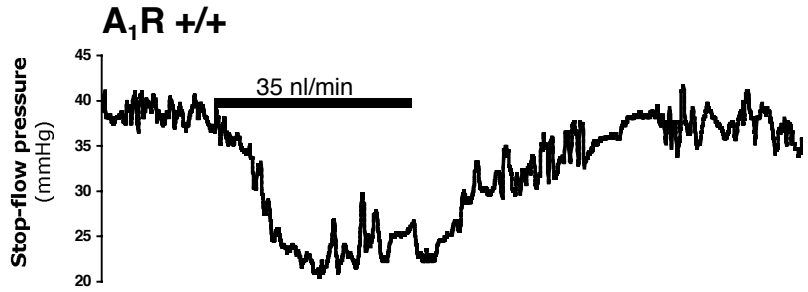

Figure 11a. *Original recording of the proximal tubule stop-flow pressure in an A_1R+/+ mouse. Distal perfusion rate is increased from 0 to 35 nl/min.*

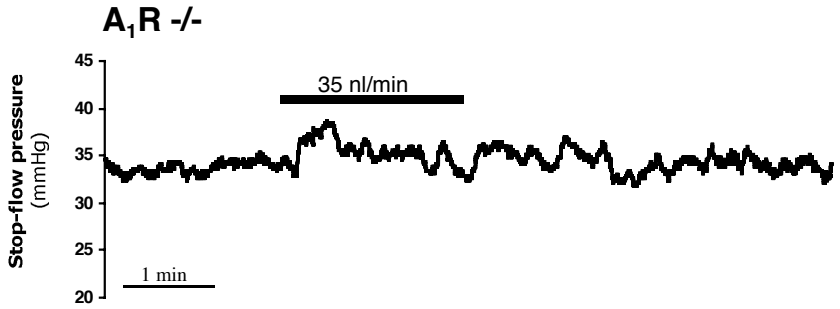

Figure 11b. *Original recording of proximal tubule stop-flow pressure in an A_1R-/- mouse. There is no TGF response, as seen as a reduction in P_{SF}, when distal perfusion rate is increased from 0 to 35 nl/min.*

The major finding in this study was the lack of TGF response in the A_1R-/- mice. The completely blocked TGF mechanism in these mice demonstrates that adenosine is an important mediator of the TGF response. The increase in plasma-renin concentration also demonstrates that adenosine has important inhibitory functions in the mechanisms for the secretion of renin from the juxtaglomerular apparatus.

Study III

The Influence of the Adenosine A_1-receptor on Blood Pressure Regulation and Renin Release

In study II we found that the blood pressure in the A_1R-/- mice was elevated. Those blood pressure measurements were done on unconscious animals and could be a consequence of anesthesia or stress causes by handling and surgical procedures. Therefore, telemetric blood pressure probes were used to monitor blood pressure in the conscious, unstrained and as much as possible non-stressed mice (Fig. 12). Plasma-renin concentration and urinary solute excretion was also measured in conscious animals on salt-deficient (SD), normal-salt (NS) and high-salt (HS) diets.

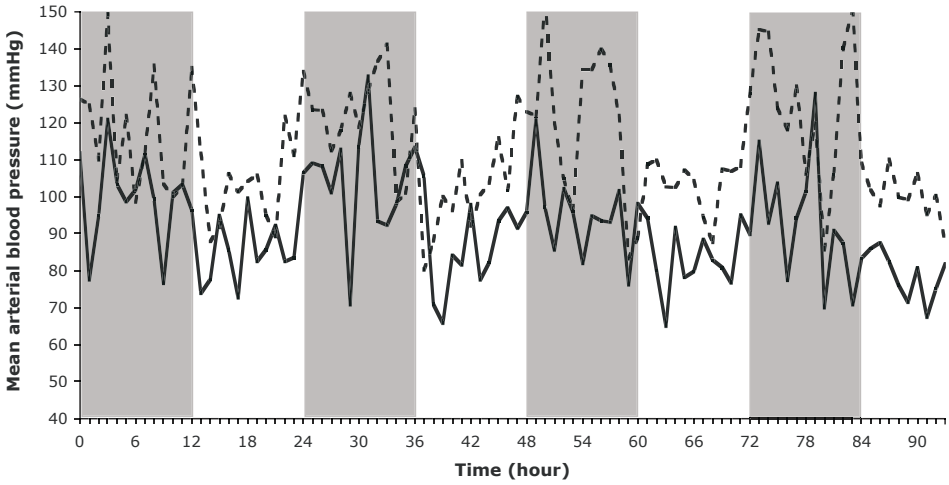

Figure 12. *Telemetric blood pressure recordings of a single A_1R+/+ (solid line) and A_1R-/- (dashed line) mouse on a normal-salt diet. Typical circadian could be observed, with a higher blood pressure during the active (nighttime; shaded areas) periods.*

A_1R-/- had an elevated blood pressure compared to A_1R+/+ except on HS-diet. On the HS-diet the A_1R+/+ blood pressure increased to the same level as the A_1R-/- animals. Plasma-renin levels were also higher in the A_1R-/- animals compared to their wild-type littermates. However, increases in dietary salt intake elicited a reduction in plasma-renin levels in the A_1R-/- mice

(Fig. 13), supporting the concept of a tonic inhibitory role of the A_1-receptor on renin secretion.

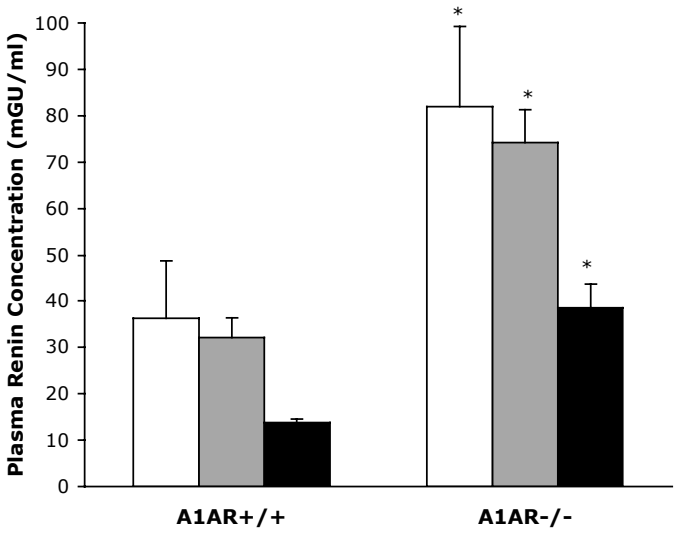

Figure 13. *Plasma renin concentrations in A_1AR+/+ and A_1AR-/- mice on salt-deficient (□), normal-salt, (▒) or high-salt, (■) diet. Values are means ± SEM. *P < 0.05 vs. A_1AR+/+ on same diet.*

Despite the elevated plasma-renin levels, the A_1R-/- mice had an increased sodium excretion. This could be both due to an abolished TGF mechanism and adenosine's direct effect on tubular sodium reabsorption, mainly in the proximal tubule.

The major finding in this study was the increased blood pressure in the conscious A_1R-/- mice and that the A_1R tonically inhibits renin secretion. Adenosine, through its actions on the A_1R, plays an important role in regulating the TGF. This in turn, will effect the regulation of renin secretion, blood pressure and electrolyte balance.

Study IV

Ischemic Preconditioning does not Protect Against Renal Injury in Adenosine A_1-receptor Knockout Mice

Ischemic injury and subsequent acute renal failure is associated with morbidity and mortality. This study was designed to further understand the role of adenosine in the protection against ischemic injury. Both A_1R+/+ and A_1R-/- animals were subjected to acute renal ischemia-reperfusion and preconditioning prior to an ischemic insult. When kidney function was investigated by measuring SNGFR, both A_1R+/+ and A_1R-/- animals showed a 50% decrease after ischemia-reperfusion. When the animals were subjected to preconditioning prior to an ischemic episode, the A_1R+/+ had only a minor decrease in SNGFR (Fig. 14). Preconditioning, however, did not protect the A_1R-/- kidney function and SNGFR decreased to that found after ischemia-reperfusion.

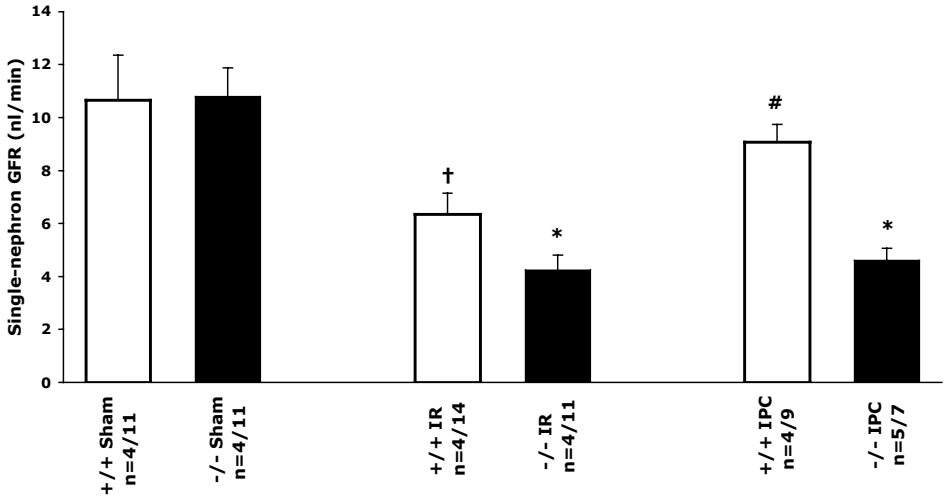

Figure 14. *SNGFR in A_1R+/+ (+/+) and A_1R-/- (-/-) mice after being either sham-operated (sham), subjected to ischemia insult (IR) or ischemic-preconditioning prior to ischemic insult (IPC). Values are given as mean ±SEM. (n = mice/tubules). *P < 0.05 vs sham A_1R-/-; †P < 0.05 vs sham A_1R+/+; #P < 0.05 vs. IR A_1R+/+.*

The protection against ischemic injury is also reflected in total kidney GFR measurements. Following ischemia-reperfusion, both genotypes show a ~25% decease in GFR. Preconditioning seemed to protect the A_1R+/+ from renal impairment, as there was only a slight decrease in GFR in these animals. In the A_1R-/- animals, however, preconditioning had no effect and GFR decreased (~25%) to the same level as was seen after ischemi-reperfusion.. Ischemic injury did not have an effect on either blood pressure or kidney weight.

The results from this study indicate that the A_1R is important in preventing ischemic damage to the kidney and that the protective effects of ischemic preconditioning are related to the presence of the adenosine A_1Rs.

DISCUSSION

One of the most basic concepts in physiology is that organ function is under metabolic control. When the metabolic rate of an organ is increased, it will produce one or more metabolites that will affect the organ's supply and/or utilization of oxygen and substrates. As seen in many organs of the body, i.e. heart, brain, skeletal muscle, the vasculature will respond with a vasodilation when the metabolic rate is increased, thus increasing blood flow and the supply of oxygen and substrates. Compared to other organs in the body, the kidneys have a very high blood flow, receiving ~20% of cardiac output under normal conditions. Since renal blood flow determines tubular fluid load and subsequent reabsorption through the glomerular filtration rate (GFR), a vasoconstrictive metabolite could reduce the workload and limit the amount of expended energy.

Adenosine and the tubuloglomerular feedback mechanism

In the 1980's, Osswald and colleagues hypothesized that the renal hemodynamics were under metabolic control of local blood flow and suggested a coupling between the metabolic rate in the macula densa (MD) cells and the release of adenosine (Osswald et al., 1982). The MD cells are situated to detect tubular fluid NaCl concentrations at a point where the concentration is almost entirely determined by the loop of Henle flow rate. According to Osswald's theory, changes in the salt concentration at the MD site correspond with changes in metabolic rate of the MD cells. Increasing distal delivery, as seen during increased distal tubular flow-rate, corresponds with an increased transport of Na^+ through the MD cells and an increased ATP utilization by the basolateral Na-K-ATPase. This will increase the demand for ATP in these cells, leading to the hydrolysis of ATP to ADP and further on to adenosine (Thomson et al., 2000). Adenosine can then diffuse into the renal interstitium, elevate the interstitial adenosine concentration around the afferent arterioles and elicit vasoconstriction.

Adenosine, through the activation of adenosine A_1-receptors, has been implicated in the final step of TGF activation and has been proposed to be the mediator of the TGF system (Brown et al., 2001; Osswald et al., 1980; Schnermann et al., 1990; Sun et al., 2001; Thomson et al., 2000).

It has been earlier shown that adenosine causes a strong vasoconstriction in the kidney in contrast to the vasodilation that is normally seen in other organs (Haas and Osswald, 1981; Hedqvist and Fredholm, 1976). The effects of adenosine on the renal vasculature are not solely from the effects by A_1R stimulation but also from adenosine A_2-receptor stimulation (Silldorff and Pallone, 2001). In most vascular beds there is a larger concentration of adenosine A_2-receptors, but in the afferent arterioles of the kidney the A_1R predominates, which produces a vasoconstriction upon stimulation from adenosine (Tang et al., 1999). Agmon demonstrated that adenosine-mediated reduction of cortical and medullary blood flow was mediated by the A_1R by specifically stimulating the A_1R with a selective A_1R agonist (Agmon, 1993). Studies in the isolated afferent arterioles have also shown that adenosine constricts the vessels and that he vasoconstriction is most pronounced in the distal region of the afferent arteriole, closest to the glomerulus (Joyner et al., 1988; Weihprecht et al., 1992). Within this region of the afferent arteriole, TGF-activated vasoconstriction is known to occur (Moore and Casellas, 1990).

As described earlier, the TGF mechanism operates by sensing the distal load to the MD cells and adjusting afferent arteriole tone and the rate of renin secretion. The sensing step involves the detection of the NaCl concentration through the Na-K-2Cl co-transport mechanism (Persson et al., 1991). Subsequently, the metabolic rate increases and adenosine is generated.

In study II we investigated the effects that adenosine A_1-receptor deficiency had on the tubuloglomerular feedback response in newly developed transgenic mouse strain, lacking the A_1R. We could clearly demonstrate the absence of a TGF response in these mice. When the loop of Henle perfusion rate was increased to 35 nl/min, there was no detectable drop in P_{SF}. This finding is completely in line with the original hypothesis made by Osswald (1982), where adenosine is perceived as a mediator of the TGF mechanism

rather than as a modulator. From a modulator one would not expect the response to be completely absent.

For a substance to be a mediator of the TGF there are certain criteria that must be met. First, its activity must be immediately altered by changes of NaCl delivery to the MD site. Second, it must be generated locally in the juxtaglomerular apparatus. Third, it must have an inhibitory action on renin secretion. Adenosine seems to fill all of these requirements for a mediator of the TGF mechanism. NaCl concentration is sensed by the Na-K-2Cl co-transport mechanism in the MD cells causing an increase in adenosine. Once in the interstitium adenosine also has a short half-life of a few seconds, resulting in reduction of adenosine activity when NaCl levels fall. As part of the TGF response adenosine also inhibits renin secretion. The results from study I also show that the actions of adenosine on the TGF are transmitted through the A_1R.

Adenosine and renin release

Renin secretion is influenced by a number of different factors, such as changes in sympathetic tone, circulating catecholamines, changes in baroreceptor activity and hormonal systems. Adenosine, acting through the A_1R has an important role in modulating the release of renin from juxtaglomerular cells. A great number of *in vivo* and *in vitro* studies have demonstrated that adenosine and A_1R agonists generally attenuate the release of renin (Churchill and Churchill, 1985; Kurtz et al., 1988; Lorenz et al., 1993; Osswald et al., 1978; Skott and Baumbach, 1985), whereas antagonism of A_1Rs, on the other hand, augment renin release (Pfeifer et al., 1995). It has also been shown that adenosine, released from the MD cells, activates A_1Rs, causing a decrease in renin release (Itoh et al., 1985).

Renin release from the juxtaglomerular granular cells is stimulated by adenylyl cyclase (Jackson, 1991). Stimulation of adenylyl cyclase causes a diffusion of intracellular cyclic AMP out of the cell (Barber and Butcher, 1981). If ectophosphodiesterase is present, then cyclic AMP could be metabolized to AMP and further to adenosine by ecto-nucleotidase. Mi and Jackson have shown that extra cellular cyclic AMP can be metabolized to adenosine through this pathway (Mi and Jackson, 1995). Since the A_1R is

negatively coupled to adenylyl cyclase by inhibitory G-proteins, lack of activation of the A_1R could increase renin secretion.

As mentioned earlier, decreased electrolyte uptake and transport from the MD cells will decrease ATP metabolism and formation of adenosine. The lower concentration of adenosine in the interstitium will decrease vascular tone, by reducing intracellular calcium in the smooth muscle cells and at the same time diminish the inhibition of renin release from the granular cells of the afferent arteriole. An increased intra cellular calcium concentration in the renin granular cells of the afferent arteriole acts as a strong inhibitor of renin release. Increases in intra cellular calcium have been shown to occur in the smooth muscle cells of the afferent arteriole upon the application of adenosine (Gutierrez et al., 1999). This increase in smooth muscle cell calcium occurred both in the proximal and distal portions of the afferent arteriole. The smooth muscle cells are responsible for the contractile response of the TGF mechanism. Furthermore, the renin producing granular cells are located in the distal part of the arteriole, where increases in calcium concentration was found. Adenosine, formed by the cyclic AMP pathway or from the hydrolysis of ATP, can affect renin secretion.

This lack of inhibition on renin release is completely in line with our findings in study II and III. We found in both studies that the mice lacking the A_1R had an increased plasma-renin concentration. To further evaluate the role of adenosine in MD-mediated renin release, mice were put on different salt diets. Changes in salt intake elicited inverse changes in plasma-renin concentration in both the $A_1R+/+$ and animals even though the $A_1R–/–$ had much higher plasma renin levels. These findings support a tonic inhibitory role for adenosine and the A_1R on renin release.

Adenosine and arterial blood pressure

Hypertension is a common human disorder, affecting over 20% of the population in the western world. There are very few recognizable and surgically treatable causes of hypertension and a majority of the cases involve no causative factor, but the pathophysiology is intimately related to the kidneys. Studies have demonstrated by transplantation experiments in which kidneys

are transplanted from or to animals with genetic hypertension, that the hypertension "travels with the kidney" from a hypertensive donor or vice versa (Heller et al., 1993). One of the major physiological mechanisms that control arterial blood pressure and that is a target for many antihypertensive drugs is the renin-angiotensin-aldosterone system. In study II we found that the arterial blood pressure was elevated in the A_1R-/- mice. This increase could be caused by the anesthesia used in the study. So, in study III the mice were implanted with telemetric blood pressure devices. This enabled blood pressure measurements to be taken without the effects of anesthesia and stress related to handling and surgery. We found that the arterial blood pressure in the conscious mice was also elevated compared to their A_1R+/+ littermates. Other studies have shown that chronic blockade of adenosine receptors with the non-selective antagonist DPSPX, leads to an activation of the renin-angiotensin system, raising the plasma levels of renin and causing an increase in blood pressure (Morato et al., 2002; Sousa et al., 2002). Guimaraes showed that the systolic blood pressure could increase as much as 40 mmHg with chronic adenosine receptor blockade (Guimaraes and Albino-Teixeira, 1996). As in these earlier studies with adenosine receptor blockers, the A_1R-/- animals also had an increased plasma-renin concentration that could be responsible for the observed increase in blood pressure.

Adenosine and ischemic injury

When blood supply to an organ is occluded for a short period of time, the normal postocclusive response is hyperemia, due to the relaxation of the vasculature. The hypoxic kidney, however, responds with postocclusive vasoconstriction. Renal ischemia or hypoxia is a potent stimulant for renal production of adenosine (Osswald et al., 1977). In the normal physiological situation ATP will provide the cells with energy, by degradation in to ADP and AMP and then adenosine. These steps are oxygen-independent and can take place in an anaerobic environment. On the other hand, the regeneration of AMP to ATP is an energy-demanding process, which requires the presence of oxygen.

Adenosine has been proposed to be involved in acute renal failure based on the fact that adenosine, and specifically the A_1R, mediates functional

changes in the renal microcirculation. Because adenosine causes vasoconstriction of the renal vasculature, which leads to reduced blood flow and GFR, it was earlier viewed that adenosine's effect was detrimental to the function of the kidney following ischemia (Pflueger et al., 1995; Yao et al., 1994). Conversely, there are more recent studies that demonstrate that activation of A_1R protects against ischemic-mediated renal injury (Lee and Emala, 2000). Preconditioning, multiple brief period of ischemia and reperfusion, has been shown to give protection against subsequent prolonged ischemia (Murry et al., 1986). In study IV we found that the A_1R-/- mice had a significantly decreased renal function compared after an ischemic episode. Furthermore, preconditioning did not prevent the A_1R-/- from renal injury (diminished in GFR and SNGFR), while it protected the A_1R+/+ mice from a decrease in kidney function.

Activation of the renal A_1R produces different effects in the kidney, reduction of renal blood flow, reduced GFR and reduced and reduced solute reabsorption through the epithelial cells. These effects will decrease the metabolic rate and, thus the oxygen demand of the kidney during hypoxia. The exact mechanism involving adenosine has not yet been elucidated, but one can speculate that the internal energy stores are not rapidly depleted allowing for basal cellular processes to continue for a longer period of time.

Nitric oxide and the tubuloglomerular feedback mechanism

Even though adenosine has been found to be the mediator for the TGF mechanism, a number of different hormones and local factors are important for modulating the sensitivity and response of the TGF. In contrast to a mediator, a modulating agent does not cause the TGF response, but instead conditions the response, enhancing or diminishing it. It is known that body fluid changes are associated with differences in TGF sensitivity. During dehydration the TGF system is highly sensitive even though the filtered load is lower than normal to avoid further fluid losses (Selen et al., 1983). In the resetting process many hormones and local factors are involved; Ang II, prostaglandins, kinin, thromboxane and others (Schnermann, 1998). Nitric oxide (NO) produced from nNOS, located in the MD cells is important for setting the sensitivity of the TGF system.

Resetting of the TGF to a lower level following volume expansion, as seen in study I, is an important mechanism because the attenuation of the TGF response allows for greater distal delivery of fluid before a TGF induced reduction in GFR occurs. This adaptation of the TGF helps in facilitating a return of the extra cellular volume to a normal level. If no resetting of the TGF took place, the TGF induced reduction of GFR would hinder an adjustment of the extracellular fluid volume. In study I we found that extracellular VE attenuates the TGF response. It is known that during volume expansion sympathetic tone is reduced as well as circulating Ang II. Sympathetic stimulus and Ang II is known to enhance TGF sensitivity, while NO is known to attenuate the TGF response. When a general nitric oxide synthase (NOS) inhibitor or neuronal NOS inhibitor was given, the TGF response was restored to control levels. The resetting of the TGF could be due to an increased NO production and/or activity. However, this indicates that NO derived from MD cells is involved in the resetting of the TGF response seen after volume expansion.

CONCLUSIONS

Taken together, the results from the present studies highlight the key role that adenosine, acting through the A_1R, has in regulating the different functions of the kidney. It can be concluded from studies in transgenic mice lacking the A_1R that:

- Adenosine mediates TGF response.
- Adenosine has important inhibitory functions in the mechanisms for the secretion of renin from the juxtaglomerular apparatus.
- Adenosine tonically inhibits macula densa stimulated renin secretion.
- Increased plasma levels of renin, caused by the lack of A_1R stimulation, induce hypertension.
- Adenosine, through its effects on the TGF and epithelial tubular cells, regulates solute excretion.
- The protective effect of ischemic preconditioning is related adenosine's actions through the A_1R.

The results from volume expanded animals show that NO, derived from nNOS in the MD cells, is an important modulator of the TGF.

- A functional nitric oxide system is important in mediating normal renal responses and that an increased production of and/or sensitivity to nitric oxide during volume expansion plays an important role in the adaptive mechanisms of the TGF.

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