Renal Dysfunction and Protection in Cardiovascular Surgery

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Examinerad läkare

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ABSTRACT

***Purpose*:** This thesis is focused on studies of postoperative renal dysfunction (PRD), and on renal effects and possible prophylactic potentials of three vasoactive substances, calcitonin gene-related peptide (CGRP), atrial natriuretic peptide (ANP), and the calcium channel antagonist diltiazem.

Methods: In paper I the medical records of 1 030 consecutive cardiothoracic surgery patients were evaluated and glomerular filtration calculated from serum creatinine levels. In paper II experimental renal ischemia was induced in normal rats. Treatment with CGRP 10 or 25 pmol/kg/min was evaluated by comparing serum urea elevations with controls. In paper III ANP 7.5 pmol/kg/min was infused postoperatively in vascular surgery patients and the renal and hemodynamic effects studied. In paper IV the renal and hemodynamic effects of ANP infusion 7.5 pmol/kg/min were studied immediately postoperatively in cardiac surgery patients. In paper V the renal and hemodynamic effects of diltiazem infusion was studied in postoperative vascular surgery patients. In paper VI diltiazem treatment for 24 h in connection with cardiac surgery was compared to placebo, in patients with a preexisting moderate renal dysfunction. Iohexol clearance was measured before surgery and 5 days and 3 weeks postoperatively.

Results and Implications: The incidence of postoperative renal dysfunction (PRD) after open heart surgery was 11 %. In PRD patients, the mortality rate was 12.4 %, compared with 0.4 % in Non-PRD patients. After experimental renal ischemia, CGRP had a protective effect on renal function. The protective effect was dose related as the higher CGRP dose resulted in systemic hypotension. ANP infusion improved glomerular filtration rate, urine flow, and sodium excretion in humans in the postoperative setting, in vascular as well as cardiac surgery patients. There were no hemodynamic side effects of diltiazem treatment (bolus 0.25 – 0.28 mg/kg, followed by infusion of 1.0 –1.7 μg/kg/min). It was shown that diltiazem, in patients with preexisting renal impairment, protected and even improved their renal function after cardiac surgery. In future studies it would be of interest to further explore the renal outcome after major surgery, trauma and shock, using as protective agents both CGRP and ANP, not only separately but also in combinations including diltiazem.

Keywords: Acute Renal Failure, Anesthesia, Atrial Natriuretic Peptide, Calcitonin Gene-Related Peptide, Calcium Antagonists, Diltiazem, Heart Surgery, Postoperative Renal Dysfunction, Vascular Surgery.

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For my family

Table of Contents

Abstract

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List of Papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

I. Bergman A, Odar-Cederlöf I, Westman L, Öhqvist G. Postoperative Renal Dysfunction in Cardiac Surgery: A Follow-up Study. *Submitted for publication.*

II. Bergman A, Fält K, Odar-Cederlöf I, Westman L, Takolander R. Calcitonin Gene-Related Peptide Attenuates Experimental Ischemic Renal Failure in a Rat Model of Reversible Renal Ischemic Insult. *Renal Failure* 16:351-357, 1994.

III. Bergman A, Odar-Cederlöf I, Theodorsson E, Westman L. Renal Effects of Human Atrial Natriuretic Peptide in Patients after Major Vascular Surgery. *Acta Anaesthesiologica Scandinavica* 38:667-671, 1994.

IV. Bergman A, Odar-Cederlöf I, Westman L, Öhquist G. Effects of Human Atrial Natriuretic Peptide in Patients after Coronary Artery Bypass Surgery. *Journal of Cardiothoracic and Vascular Anesthesia* 10:490-496, 1996.

V. Bergman A, Odar-Cederlöf I, Westman L. Renal and Hemodynamic Effects of Diltiazem after Elective Major Vascular Surgery. *Renal Failure* 17:155-163, 1995.

VI. Bergman A, Odar-Cederlöf I, Westman L, Bjellerup P, Höglund P, Öhqvist G. Diltiazem Infusion for Renal Protection in Cardiac Surgery Patients with Preexisting Renal Dysfunction. *Accepted for publication by Journal of Cardiothoracic and Vascular Anesthesia.*

Abbreviations

AA	Aortic Aneurysm
ANP	Atrial Natriuretic Peptide
ARF	Acute Renal Failure
AVR	Aortic Valve Replacement
BSA	Body Surface Area
CABG	Coronary Artery Bypass Grafting
cAMP	Cyclic Adenosine Monophosphate
CGRP	Calcitonin Gene-Related Peptide
CO	Cardiac Output
CPB	Cardiopulmonary Bypass
CVP	Central Venous Pressure
F_iO_2	Fraction Inspired Oxygen
GFR	Glomerular Filtration Rate
ICU	Intensive Care Unit
IM	Intramuscular
IP	Intraperitoneal
IV	Intravenous
K_f	Glomerular Filtration Coefficient
MAP	Mean Arterial Pressure
M	Molar
MW	Molecular Weight
NS	Not Significant
PRD	Postoperative Renal Dysfunction
RBF	Renal Blood Flow
S_{Cr}	Serum Creatinine
$T^1/_2$	Half-life
VR	Valve Replacement

INTRODUCTION

The kidneys have several key roles to play for filtration of blood and excretion of metabolic products, to regulate water and electrolyte balances and to influence systemic blood pressure. The maintenance of a normal renal function is a main therapeutic target in traumatized patients, whether it is due to surgery or accidents. During World War II, the syndrome of acute renal failure (ARF) was first recognized [Bywaters 1941] and was later described in more detail [Bull 1950, Swann 1953]. Modern techniques of aggressive fluid resuscitation have strongly reduced the incidence of acute renal failure after trauma [Anderson 1997] and the concern today is mostly focused on major surgical procedures, *e.g.* implantation of synthetic vascular grafts, and the use of extracorporeal circulation, especially in patients with preexisting chronic medical conditions [Gornick 1983, Turney 1990, Schepens 1994, Kellerman 1994, Anderson 1997]. In these surgical settings, the incidence and severity of acute renal failure is still reported to be high [Byrick 1990, Kellerman 1994, Aronson 1998]despite increased vigilance. One reason for this may be a continuing development towards more high-risk patients being treated with more high-risk surgical procedures [Aronson 1998, Urzua 1999].

Severe renal hypoperfusion injury may seem a paradox, since RBF accounts for about 25 % of cardiac output, giving the kidneys one of the highest perfusion rates in the body when calculated on an organ weight basis. Additionally, there is a renal autoregulatory mechanism to protect from hypoperfusion of the kidneys even if systemic blood pressure is low. Despite this, the incidence of hypoperfusion damage is much higher in the kidneys than in other organs, *e.g.* the liver and the brain, during the same clinical conditions. The reason for this vulnerability is not entirely known. There is probably a heterogeneity of renal oxygenation, where especially parts of the outer medulla are working on the verge of ischemia under normal conditions, and consequently the risk for hypoxic cell damage is high. Anesthesia, surgery, and trauma have the capacity to challenge the balance between supply and demand of oxygen in the kidneys. A primary goal must therefore be to ascertain renal blood flow in order to support the oxygen delivery required to preserve glomerular and tubular function.

Glomerular filtration rate (GFR) is dependent on plasma flow, transcapillary hydraulic and colloid osmotic pressure differences, and the filtration coefficient [Maddox 1996]. An integral functional part of glomerular filtration is the mesangium which is no longer regarded as a supportive tissue only [Sweeney 1990] (**Figure 1**). The mesangium has a significant influence not only on the coefficient of filtration (K_f) but also, probably via contractile elements in mesangial cells, on

glomerular perfusion. These mesangial functions may be affected by several factors such as hormones, cytokines and various macroaggregates [Sweeney 1990]. For instance the formation of macroaggregates in connection with major surgery and trauma may thus initiate arteriolar preglomerular vasoconstriction. This further emphasizes the complexity of perfusion regulation and how surgical trauma may influence glomerular filtration and may therefore constitute a target mechanism for renal protection.

A major surgical procedure is to be regarded as a tissue trauma with activation of inflammatory and pro-inflammatory systems and with cytokine production [Tønnesen 1996, Lin 2000]. Exposure of the blood to artificial surfaces, *e.g.* the implantation of synthetic grafts particularly in vascular surgery, may add further injury to the blood. Furthermore, in cardiac surgery extracorporeal circulation is often practiced which also adds to the mechanical injury of circulating blood and activates neutrophils and complement factors [Herskowitz 1996]. Genetic factors, such as different isoforms of apolipoprotein E, may also influence postoperative renal function, possibly by interacting with inflammatory responses, tissue repair, or cell differentiation and growth [Chew 2000]. All these factors contribute to increase the risk for development of postoperative renal dysfunction. This condition was earlier also sometimes provoked by specific inorganic metabolites, fluorides, produced from volatile anesthetic agents. These circumstances have been elucidated in several previous publications [Crandell 1966, Mazze 1971, Järnberg 1979]. Today new, and from a renal point of view safer, inhalational anesthetic agents have been developed. However, one of the latest additions, sevoflurane, produces small amounts of fluoride metabolites and hence has a potential for renal toxicity [Kharasch 1995]. This is particularly true after prolonged anesthesia with an accumulated fluoride load and sevoflurane is not recommended for anesthesia of such cases or in patients with preoperative renal dysfunction.

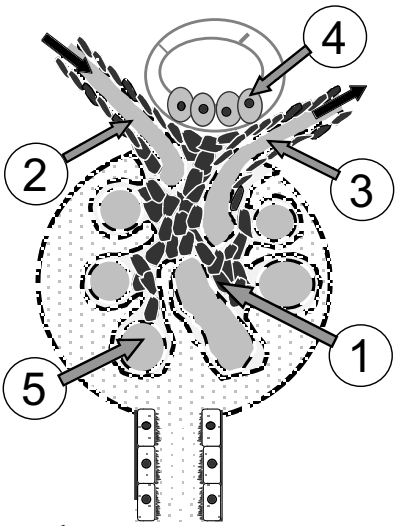


Figure 1.
Section through a glomerulus and the juxtaglomerular apparatus. The extraglomerular nongranulated mesangial (Goormaghtigh) cells are continuous with granular cells, smooth muscle cells of the arteriolar walls, and with the intraglomerular mesangial cells (1). Afferent arteriole (2). Efferent arteriole (3). Macula densa cells (4). Cross-sectioned glomerular capillaries (5).
Adapted from Hebert 1997.

Inhaled nitric oxide or aerosolized prostacyclines opened up possibilities for organ specific, in this case lung specific, effects on vascular tone [Weitzberg 1993, Santak 1995]. Systemic side effects such as hypotension were thus circumvented. In parallel, the problem with renal protection would be easier to solve if renal specific vasodilators were at hand. A relatively newly found potent vasodilator, Calcitonin Gene-Related Peptide, CGRP, is described both as a transmitter in the nervous system and as an agent with direct effects on vascular smooth muscles with a strong vasodilating action [Brain 1985, Uddman 1986]. CGRP is a 37-amino acid regulatory peptide which is released from perivascular nerve endings [Zaidi 1985] into the blood stream, where the $T^1/_2$ is approximately 10 minutes [Brasilis 1988]. In the kidney CGRP decreases renal vascular resistance, and increases renal blood flow and glomerular filtration rate [Villarreal 1988a, 1988b]. The problem is, however, organ specificity and how to avoid systemic hypotension. In theory, the rete glomeruli with its mesangium interacting with blood vessels could respond to a potent vasodilator such as CGRP at a lower dose compared with the dose required to dilate microvessels and arterioli in other vascular beds. Hitherto, relevant dose finding studies focused on renal protective effects of CGRP have not been performed. If there is a safe "renal dose" of CGRP, *i.e.* without systemic hypotensive effects, this vasoactive peptide would constitute a potent agent for renal protection.

Atrial Natriuretic Peptide (ANP) is an endogenous 28-amino acid peptide first discovered in 1981 [de Bold 1981]. It is released from the heart atria in response to atrial distension, *e.g.* due to fluid overload. In man it has a plasma $T^1/_2$ of approximately 4.5 minutes [Theodorsson 1990]. Specific ANP receptors have been identified in the glomeruli (mesangial cells), in the inner medullary and papillary collecting duct, and in vascular smooth muscle [Napier 1984]. ANP has direct vasodilating effects, antagonizes vasoconstrictive agents, and increases renal water and electrolyte excretion [Espiner 1986]. In addition, ANP has been shown to reduce energy demands of renal medullary functions such as sodium re-absorption in tubuli by deactivation of Na^+,K^+-ATPase [Clausen 1991]. This may also be an aspect to pursue with regard to renal protection by ANP in connection with surgery and trauma.

ANP is a versatile peptide, well documented not only in the animal but also in the plant kingdom [Vesely 1993, Yang 1999]. It is found in roots, stems, leaves, and flowers. Its physiological role in plants is most likely linked to water and solute transport, probably by inducing stomatal opening [Billington 1997]. A postulated mechanism for this is dilation of plant vessels which is in turn suggested to occur with cGMP as an intracellular mediator [Vesely 1993]. With documented effects of ANP on vessel diameters, solute transport, and osmotic pressures, in plants as well as in animals and humans, ANP must be regarded as an important agent involved in water and electrolyte

balance, *i.e.* in human renal function. Its use as a protective and therapeutic agent in acute renal failure has been investigated by several researchers [Ratcliffe 1991, Sands 1991, Valsson 1996, Allgren 1997]. The results have, so far, not been conclusive and the matter is still open to debate [Brenner 1997, Humes 1997, Allgren 1998, Galley 2000].

Increased cytosolic calcium concentrations are involved in vasoconstriction *via* effects not only on vascular smooth muscle but also on contractile elements in the mesangium [Greger 1996]. Accordingly, blocking of the calcium channel function has been suggested as another therapeutic strategy for a maintained glomerular function [Neumayer 1991]. Calcium channel blockers, widely used for hypertension, angina pectoris, and cardiac arrhythmias, also have important effects on renal vasculature and tubuli [Chan 1990]. The renal vasodilatory effects of calcium antagonists depend not only on the degree of vasoconstriction but also on the mechanism thereof and the preglomerular vessels are sensitive to calcium antagonists, resulting in an enhanced GFR [Loutzenhiser 1985]. Free radical formation and interference with mesangial macromolecular entrapment have been reduced by using calcium antagonists [Sweeney 1990, Neumayer 1991, Epstein 1992b]. Furthermore, calcium antagonists enhance sodium and water excretion, probably *via* a direct influence on tubular function [Wetzels 1988], and reduce aldosterone secretion [Osswald 1990]. In addition, calcium antagonists have been associated with cyto-protective effects in acute renal failure by inhibition of increased intracellular calcium load related to an ischemic renal insult [Chan 1990]. Some differences exist between the different calcium channel blockers and diltiazem has been reported to have minimal effects on cardiac conduction and contraction as well as on systemic vascular resistance [Kates 1987]. Diltiazem is a benzothiazepine derivative and has been extensively investigated both for organ protection in connection with renal transplantation [Alcaraz 1991, Neumayer 1992] and for myocardial protection in cardiac surgery [Donegani 1986, Seitelberger 1994]. The plasma $T^1/_2$ of diltiazem is reported to be 2 – 5 hours [Buckley 1990].

In summary, there are several factors during major surgery and trauma, that by themselves or in combination may cause postoperative renal dysfunction (PRD). To identify and avoid these factors is an important task. It is of value to find the magnitude of PRD in connection with major cardiovascular surgery using modern techniques. It is also important to find the best strategy for renal protection in situations where this is deemed necessary. The main purposes of this series of investigations were to describe the incidence of PRD and to shed some light on the prophylactic possibilities for prevention of PRD by the study of three different pharmacological agents, CGRP, ANP, and the calcium channel blocker diltiazem.

Aims of the Thesis

The overall objective of this thesis was to find a strategy for renal protection in patients subjected to cardiovascular surgical procedures. Specific aims were to:

* describe the current incidence of postoperative renal dysfunction (PRD), in connection with cardiothoracic surgery

* analyze, experimentally, if different doses of a potent vasodilator, Calcitonin Gene-Related Peptide (CGRP), had a nephroprotective potential

* find out if an infusion of Atrial Natriuretic Peptide (ANP) improved renal function on postoperative day 1 in patients subjected to abdominal aortic surgery using infrarenal aortic crossclamp

* evaluate the effect of an ANP infusion on renal function immediately after coronary artery bypass surgery

* assess the effects on hemodynamics and renal function of a calcium channel blocker, diltiazem, in patients after abdominal aortic surgery

* elucidate renal protective effects of calcium channel antagonism using diltiazem in patients with preexisting renal impairment undergoing open heart surgery.

Materials and Methods

Patients

In **paper I**, the medical records of 1030 consecutive patients were investigated. All patients had cardiothoracic surgery using cardiopulmonary bypass. The types of operations performed are given in **Table 1**. Postoperatively the patients were cared for in the Cardiothoracic ICU.

Seven patients in **paper III** and ten in **paper V**, respectively, were studied postoperatively on the day after uncomplicated vascular surgery with insertion of an aortobifemoral synthetic vascular graft. Preoperative renal function was normal. At the time of the study, they were all breathing spontaneously and the systemic circulation had been stable for at least 4 h.

In **paper IV**, the investigations were carried out in 30 patients, immediately after uncomplicated coronary artery bypass surgery. Preoperative renal function was normal. During the study, the patients were still sedated and mechanically ventilated. The patients were randomized to double blind infusions of ANP or placebo

In **paper VI**, patients who had laboratory evidence of moderately impaired preoperative renal function were selected, *i.e.* serum creatinine in the range 120 – 300 μmol/L. Twenty-four patients were studied in connection with cardiac surgery, of which 19 had solitary coronary artery bypass grafting (CABG), 2 aortic valve replacement (AVR), and 3 had combined procedures (CABG + VR).The patients were

Table 1. Type of surgical procedure and number of patients in Paper I.

Surgical procedure	*No. of patients*
CABG	713
CABG + VR	111
VR	148
AA	33
Other	25
Total	1 030

CABG = coronary artery bypass grafting,
VR = valve replacement,
AA = aortic aneurysm repair.

randomized to double blind infusions of diltiazem or placebo. In all other aspects, the patients were treated according to the standard clinical protocols of the hospital.

Animals

Fifty-two male adult Sprague-Dawley rats were evaluated in **paper II**. They showed no signs of disease and their body weights varied between 328 – 422 g. The rats were randomly assigned to receive CGRP in a dose of 10, or 25 pmol/kg/min, or vehicle alone.

Anesthesia and Analgesia

Patients investigated in **papers I, IV** and **VI** all underwent cardiothoracic surgery. Routine anesthetic management included continuation of oral maintenance medical treatment for angina and hypertension, *e.g.* beta blocking agents, calcium antagonists, ACE inhibitors, digitalis, and nitrates, till the morning of surgery. Premedication consisted of intramuscular morphine (5 – 15 mg) and scopolamine (0.2 – 0.6 mg). Antibiotic prophylaxis with sodium cloxacillin or cefuroxime was given peroperatively. Blood pressure was monitored *via* a radial artery catheter, ECG was used to monitor cardiac rhythm and ST-changes, and a catheter was placed in the urinary bladder. Transesophageal echocardiography was used in all patients. Anesthesia was induced with fentanyl 10 µg/kg and midazolam 70 µg/kg followed by an infusion of fentanyl 5 µg/kg/h and midazolam 50 µg/kg/h. In **papers I** and **VI** 0.5 – 1 % isoflurane was sometimes used for supplementation. Muscle relaxation was achieved using pancuronium 0.1 mg/kg. The patients were mechanically ventilated with oxygen and air (F_iO_2 0.4 – 1.0) to reach normocapnia and arterial oxygen saturation of 95 % to 100 %. After surgery, most patients were mechanically ventilated in the ICU for 3 – 6 hours. Infusions of ketobemidone 1 – 3 mg/h were used for postoperative analgesia and in **paper IV** propofol 60 – 100 mg/h for sedation.

The animals in **paper II** were allowed standard rat chow and water *ad libitum* before and after the experiments. Anesthesia was induced by IP injection of pentobarbital (48 mg/kg) and maintained by additional doses 1/3 thereof. IV infusions of 0.2 % albumin solution were given at a rate of 5 mL/kg/h. The rats were kept at a constant temperature during the experiments, after which they were allowed to recover and returned to their cages. In a separate series of experiments, blood pressure was recorded *via* an arterial line inserted in a ligated femoral artery. These experiments were conducted in the same manner except that these rats were sacrificed immediately after the experiments.

Vascular surgery patients in **papers III** and **V** were studied the day after surgery performed under general anesthesia (thiopentone – isoflurane – fentanyl) in combination with epidural analgesia using intermittent bupi-

vacain. At the time of the study they were receiving intermittent IV injections of morphine 5 mg and midazolam 2.5 mg for pain relief and sedation, respectively, while no epidural injections had been given for at least 4 hours.

Investigational Methods

Paper I

Data were retrieved from the hospital records by one investigator according to a pre-determined form, transferred to a computerized data base, and statistically evaluated.

Serum creatinine concentrations (S_{Cr}) were routinely sampled prior to surgery, and on postoperative days 1, 3, and 5, and were analyzed by an enzymatic method (Vitros Instrument, Johnson & Johnson Clinical Diagnostics, Inc., Rochester, NY, USA). In those patients where a continued renal follow-up was considered necessary, S_{Cr} was measured on a daily basis.

Glomerular filtration rate was estimated from serum creatinine using the Cockroft – Gault formula:

$$GFR = K * \frac{(140 - age) * BodyWeight}{S_{Cr}}$$

where GFR is given in mL/min when age is given in years, body weight in kilograms, and S_{Cr} in µmol/L. The constant *K* is 1.23 for men and 15% less (0.85 * *K*) for women [Cockroft 1976]. GFR was normalized to 1.73 m^2 BSA [Isaksson 1958].

Postoperative renal dysfunction was defined as:

1. postoperative hemodialysis *de novo*,
2. a GFR reduction by at least 33% (equivalent to a 50% S_{Cr} increase), or
3. a GFR reduction by at least 25% if a lowest GFR < 30 mL/ min /1.73m^2 was noted, indicating a loss of renal function to such an extent that secondary complications such as anemia, and imbalances of electrolytes, calcium, phosphate, and acid-base, may appear.

Paper II

Renal ischemia was induced by clamping, *via* a midline laparotomy, the carefully dissected renal arteries with arterial clamps for 40 min. The ensuing renal impairment was quantified by comparing serum urea levels on day 1, 3, and 7 to baseline. Serum urea was assayed colorimetrically, using a commercial kit (Kodak Ektachem, Eastman Kodak Co., Rochester, NY, USA).

Papers III, IV and V

Glomerular filtration rate and ***renal plasma flow*** were determined with a standard clearance technique adapted from Smith [Smith 1956]. A priming dose of 0.5 mL/kg of a solution containing 85 mg/mL of inulin (Inutest, Laevosan-Gesellschaft, Mainz, Austria) and 30 mg/mL paraamino hippuric acid (PAH, Aminohippurate Sodium, Merck, Sharp & Dohme, West Point, PA, USA) in 5% glucose was given intravenously. This was followed by an infusion throughout the study, set at a rate of

0.5 mL/min and carried out by a motor pump (IMED, Oxon, UK). Equilibration time before the start of the investigation was one hour. Urine was collected *via* an indwelling catheter, the bladder being emptied by air insufflation and aspiration. The urine volume was measured using graduated cylinders. Inulin was analyzed according to Heyrovsky [Heyrovsky 1956] and paraamino hippuric acid according to Brun [Brun 1951]. Values for clearance of inulin (C_{In}) and para-amino hippuric acid (C_{PAH}) were normalized to 1.73 m^2 BSA.

Sodium concentrations were analyzed by direct flame photometry (IL-43, Instrumentation Laboratory, Lexington, MA, USA). An osmometer (Knauer, Berlin, Germany) was used to measure ***osmolality***. ***Chloride*** concentrations were measured in an automatic chloride analyser (Aminco-Cotlove, American Instruments Co., Silverspring, MD, USA). The equation $C_x/C_{In} = 100*C_x/C_{In}$ was used to calculate fractional clearance of a substance *x* expressed in percent of GFR, where C_x is clearance of *x* and C_{In} is inulin clearance in mL/min, respectively.

Plasma levels of **Atrial Natriuretic Peptide** were determined in **papers III** and **IV** by means of competitive radio-immunoassays (RIA) in plasma samples extracted using SepPak [Berglund 1991].

Paper VI

GFR was estimated from calculations of ***iohexol clearance*** according to Krutzén [Krutzén 1984] and normalized to 1.73 m^2 BSA. Serum concentrations of iohexol were measured with reversed-phase high-performance liquid chromatography. Serum was diluted 1:1 by water and 5 µL were injected directly onto an octadecylsilylsilica (C-18) column (250x4.8 mm). Iohexol was eluted with a mobile phase containing 0.1 % phosphoric acid and 0.2 to 4 % methanol (depending on the manufacturer of the column) at a flow rate of 1 mL/min. Eluted iohexol was identified and quantified by absorbance at 254 nm. The coefficient of variation for the iohexol clearance technique is < 5 %.

Investigated Agents

Calcitonin Gene-Related Peptide (CGRP)

In **paper II**, synthetic rat CGRP (MW 3806.94) from Peninsula Laboratories (Belmont, CA, USA) was dissolved in an 0.1 M acetic acid solution before being added to the IV infusion (0.2 % albumin in normal saline). The administration rates used were 10 (CGRP10 group) and 25 (CGRP25 group) pmol/kg/min, respectively, and vehicle alone in the control group (C).

Atrial Natriuretic Peptide (ANP)

The synthetic human ANP used in **paper III,** was purchased from Bissendorf Peptide GmbH, Wedemark, Germany (hANaP-Bissendorf [1-28], MW = 3030), and in **paper IV** from Peninsula Laboratories Europe, St. Helens, UK (α-hANP [1-28], MW = 3078.45). The respective substances were dissolved in an 0.5% solution of human albumin in isotonic saline and

sterilized by filtration at the hospital pharmacy. The infusion rate used was 7.5 pmol/kg/min.

Diltiazem

In **papers V** and **VI,** diltiazemhydrochloride for parenteral administration (Dilzem®, Gödecke AG, Berlin, Germany) was used. The substance was dissolved at the hospital pharmacy in accordance with the instructions from the manufacturer.

Statistics

The results were presented either as means ± standard deviation (**paper I)**, means ± standard error of the means (**papers II** and **IV**), or medians and 25th and 75th percentiles (**papers I, III**, **V**, and **VI**). The level of statistical significance was set to $p< 0.05$ in all papers. The Bonferroni method of correction for multiple comparisons [Bland 1995] was applied where required in **paper VI**.

The statistical methods included Student´s t-test (**papers I**, **II** and **IV**), the Fischer exact test (**paper VI**), the Chi-squared test (**papers I** and **IV**) with Yates' correction applied when expected frequencies were < 10 (**paper I**), and the Mann-Whitney U test (**papers I** and **VI**). The Wilcoxon matched pairs (sign rank) test was used (**papers I, III** and **IV**). ANOVA for repeated measures (**papers IV** and **II**) was followed by Duncan´s range test in **paper II**. In **paper IV** the respective changes from baseline were analyzed, in order to compensate for a skewed randomization. In **papers III** and **V**, Friedman two-way ANOVA followed by multiple comparisons [Siegel 1988], was used. The biochemical data in **paper VI** was analyzed using Procedure Mixed in SAS [Littell 1996]. The model was set up as a repeated measures design. Different covariance pattern models were tested, compound symmetry, first order autoregressive, and Toeplitz (general autoregressive model). Treatment, time, and treatment *x* time effects were fitted as fixed effects. Treatment effect was the between factor (diltiazem, placebo) and time effect was the within factor (3, 4, 5, or 6 time points, respectively).

Considerations on Ethics

The protocols of all human studies (**papers I, III, IV, V,** and **VI**) were evaluated and approved in advance by the Ethics Committee of the Karolinska Hospital. The studies were planned and performed in accordance with the principles of the Declaration of Helsinki 1975. The patients who participated in the studies in **papers III – VI** were informed, orally as well as by an information leaflet, and gave their consent to participation before inclusion. The Regional Ethics Committee on Animal Research evaluated and approved in advance the protocol for the animal study in **paper II**. These experiments were carried out in a special facility for animal research where the animals were accomodated and cared for by specially trained personnel.

There were no procedure-related complications during the studies.

RESULTS

Paper I

In the whole material, PRD occurred in 113 patients (11.0 %). Coronary artery bypass surgery (CABG) was performed in 713 patients with a PRD incidence of 9.1 %. Patients subjected to combined surgery with valve replacement (VR) and CABG (n = 111) had a PRD incidence of 20.7 % ($p < 0.001$, compared with CABG patients). The highest PRD rate, 24.2 %, was found in the 33 patients who had surgery for thoracic aortic aneurysms ($p < 0.05$, compared with CABG patients) (**Figure 2**). There were altogether 18 patients who died in hospital (1.7%), of which 14 in the PRD group (12.4 %). Eleven patients (1.1 % of the whole population) needed postoperative hemodialysis *de novo*. Patients who developed PRD stayed longer in the ICU (3.7 *vs.* 1.5 days, $p < 0.001$) as well as in the hospital (12.4 *vs.* 8.7 days, $p < 0.001$), (**Figure 3**).

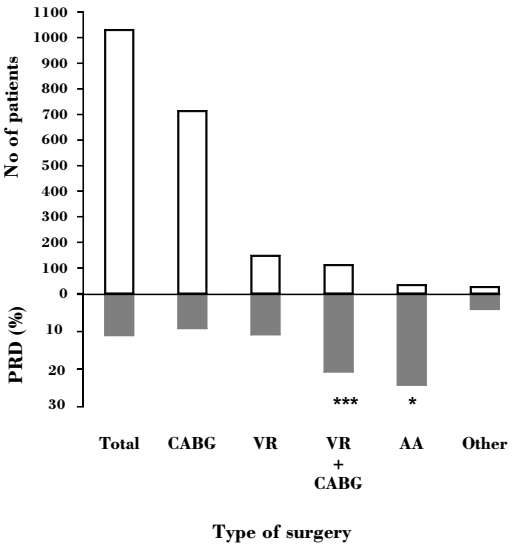


Figure 2. *Cardiac surgery and postoperative renal dysfunction. Number of patients (open bars) and incidence of PRD (shaded bars) in all patients (Total), and in different patient groups according to surgical intervention. CABG = coronary artery bypass grafting, VR = valve replacement, VR+CABG = combined procedure, AA = operation for thoracic aortic aneurysm. Asterisks (***) and (*) indicate p-values of < 0.001 and < 0.05, respectively, compared with the CABG group.*

Paper II

Serum urea levels reached a peak after 24 h in all groups. They were significantly lower at all time points after ischemia in the two CGRP treated groups, compared with the control group. Mean serum urea levels returned to baseline levels from day 3 in the rats given CGRP 10 pmol/kg/min. In the rats receiving CGRP 25 pmol/kg/min, however, as well as in control animals, they remained elevated from baseline levels throughout the study period (**Figure 4**). Mean arterial pressures (MAP) during 2 h following ischemia were significantly different in the three groups ($p < 0.001$). Compared with baseline measurements, MAP varied between 75 - 90%, 75 – 80%, and 50 - 60%, respectively, in the control, CGRP10 and CGRP25 group (**Figure 5**).

Paper III

Plasma ANP levels increased from 50 to 250 pmol/L during ANP infusion and decreased after discontinuance to a level equal to baseline. During the three 20 min periods of ANP infusion, glomerular filtration rate increased by 58%, 20%, and 21%, respectively, from a baseline level of 92 mL/min. Renal plasma flow was unchanged. Urine flow rate increased from a baseline of 1.99 mL/min by 81%, 151%, and 173%, respectively, during the same periods. Fractional clearances of sodium, chloride and osmoles were increased from baseline during the second and third ANP periods (**Table 2**). There were no changes in catecholamine levels or plasma renin activity during the study. Heart rate, mean arterial pressure and calculated systemic and

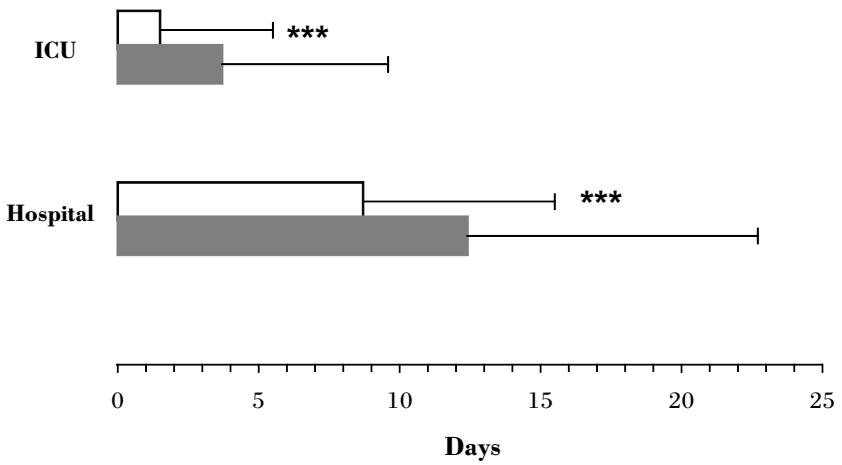


Figure 3. *Days spent in the intensive care unit (ICU) and total hospital stay. Means + SD. Open bars Non-PRD, filled bars PRD group. Compared with the Non-PRD group, *** indicates $p < 0.001$.*

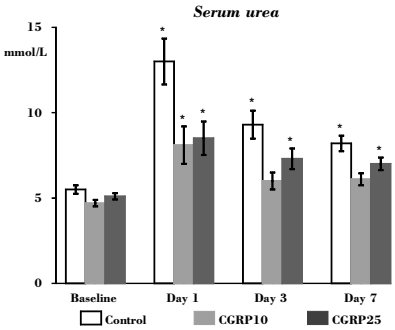


Figure 4.
Serum urea levels (means ± SEM) before and on day 1, 3 and 7 after 40 min of bilateral renal artery occlusion in the untreated control group (n=18) and after treatment with CGRP at 10 pmol/kg/min (CGRP10 group, n=8) and 25 pmol/kg/min (CGRP25 group, n=10), respectively. Asterisk (*) denotes p< 0.05 compared with baseline determination in the same group.

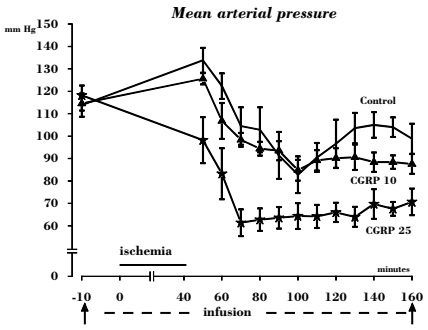


Figure 5.
Mean arterial pressure (MAP) before and after 40 min of bilateral renal artery occlusion in the untreated control group (n=6), during treatment with CGRP at 10 pmol/kg/min (CGRP10 group, n=5) and 25 pmol/kg/min (CGRP25 group, n=5), respectively. Means ± SEM.

Table 2. Renal variables during ANP infusion in Paper III.

	UF	C_{In}	C_{Nd}/C_{In}	C_{Osm}/C_{In}	C_{Cl}/C_{In}
	mL/min	*mL/min/1.73m²*	*%*	*%*	*%*
Control	1.99 (0.96 - 2.08)	92 (73 - 100)	2.08 (0.61 - 2.87)	3.20 (2.33 - 5.07)	2.01 (0.73 - 3.55)
ANP I	3.60 * (1.80 - 7.25)	125 * (100 – 138)	3.13 (0.75 - 5.50)	4.74 (2.34 - 7.48)	3.56 (0.85 - 6.45)
ANP II	5.00 * (1.55 - 7.80)	99 * (76 - 121)	3.26 * (1.05 – 7.05)	5.08 * (2.49 – 9.18)	4.85 * (1.21 – 8.68)
ANP III	5.45 * (1.18 - 8.18)	109 * (92 - 113)	5.38 * (1.09 - 7.31)	6.69 * (2.50 – 9.58)	5.67 * (1.23 - 9.06)
ANOVA, p	*0.01*	*0.01*	*0.01*	*0.02*	*0.01*

Urine flow rate (UF), inulin clearance (C_{In}), fractional clearance of sodium (C_{Na}/C_{In}), chloride (C_{Cl}/C_{In}), and of osmoles (C_{Osm}/C_{In}). ANP I, II, and III are 20 min periods of ANP infusion. Asterisk (*) denotes p< 0.05 compared to control measurement when tested with Friedman two-way ANOVA followed by multiple comparisons.
Medians, 25th - 75th percentiles

pulmonary vascular resistance did not change whereas reductions occurred in cardiac index, mean pulmonary artery pressure, pulmonary artery wedge pressure and mean right atrial pressure.

Paper IV

Plasma ANP levels increased nearly ten-fold from a mean of 7.0 ± 1.1 pmol/L in those patients who received ANP and remained at baseline levels in controls ($p < 0.001$). In the ANP group, there was a significant increase in urine flow ($p < 0.001$), inulin clearance ($p < 0.001$), and filtration fraction ($p = 0.007$), and in fractional clearance of sodium ($p < 0.001$) and of osmoles ($p < 0.001$), compared with controls (**Figure 6**). During the study, no differences in mean arterial pressure, heart rate, and right atrial or pulmonary capillary wedge pressure were detected between the groups. Cardiac index decreased by 5 % in the ANP group compared to a 9 % increase in controls ($p = 0.027$). Vasopressin levels significantly increased in controls but remained at baseline levels in ANP treated patients ($p = 0.031$). There were no changes in levels of plasma concentrations of catecholamines or angiotensin II.

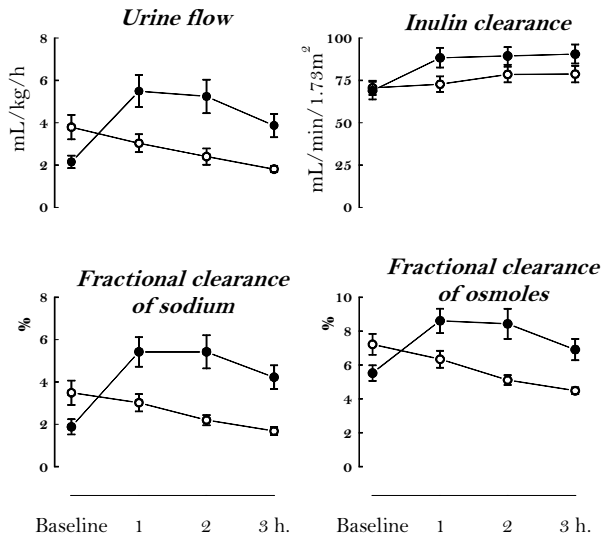


Figure 6. *Urine flow, inulin clearance, and fractional clearances of sodium and of osmoles in Paper IV. Open symbols are controls and filled symbols ANP patients. In the ANP group, all variables were significantly elevated from baseline ($p < 0.001$) and when compared with controls ($p < 0.001$) during the 3 h infusion of ANP. Means ± SEM.*

Paper V

GFR (inulin clearance) increased slightly during the initial period of DTZ infusion (**Table 3**) and the mean heart rate decreased from 84 to 77 beats per minute (p= 0.04). There were no changes during the study period in any of the other variables, compared to baseline, such as systemic hemodynamics, urine flow, renal plasma flow, fractional excretion of electrolytes and osmoles, and serum concentrations of catecholamines.

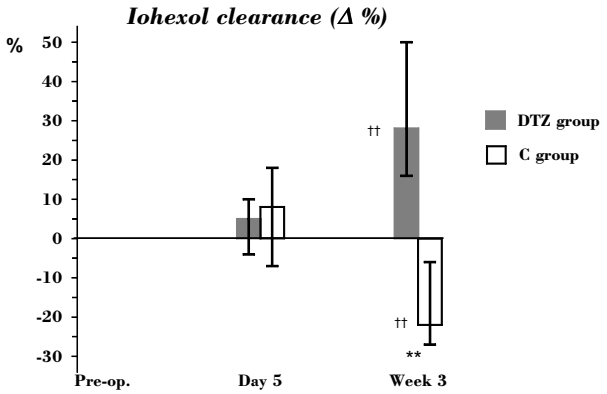
Paper VI

The postoperative changes in levels of iohexol clearance (**Figure 7**) did not differ between the groups on day 5, but was higher in the DTZ than in the placebo group 3 weeks after surgery (median 51 *vs.* 40 mL/min/1.73m^2, p< 0.05). At this time point, iohexol clearance was increased in the DTZ group (p< 0.01) and decreased in controls (p< 0.01), compared with baseline levels in the same group. Urinary N-acetyl-β-glucosamidase (U-NAG) concentrations were similar between the groups during the study but varied over time with an increase from baseline on day 2 and 4, and 3 weeks postoperatively (**Figure 8**).

Table 3. Hemodynamic and renal variables during diltiazem infusion in Paper V.

	HR *b/min*	***MAP*** *mm Hg*	***UF*** *mL/min*	C_{In} *mL/min/1.73m²*	C_{PAH} *mL/min/1.73m²*
Control	84 (81 - 86)	87 (79 - 104)	1.22 (0.99 - 1.54)	84 (80 - 97)	448 (398 - 595)
DTZ I	77 (73 - 80)	84 (74 - 94)	1.13 (0.80 - 1.74)	100 (80 - 114)	560 (386 - 628)
DTZ II	76 * (74 - 80)	87 (80 - 93)	1.03 (0.83 - 1.60)	95 (74 - 100)	426 (384 - 529)
DTZ III	77 * (74 - 79)	86 (78 - 90)	1.09 (0.85 - 1.34)	91 (75 - 112)	406 (366 - 552)
ANOVA, p	*0.04*	*n.s.*	*n.s.*	*n.s.*	*n.s.*

Heart rate (HR), mean arterial pressure (MAP), urine flow (UF), and inulin (C_{In}), and paraamino hippuric acid (C_{PAH}) clearances, before (Control), and during three 20 min periods of diltiazem infusion (I, II, III). Asterisk (*) denotes p< 0.05 for comparisons with control measurements, tested with ANOVA followed by multiple comparisons.
Medians, 25th – 75th percentiles.



	Pre-op.	Day 5	Week 3 Postop.
DTZ	39 (32 - 47)	44 (32 - 51)	51 (42 - 59)
C	47 (41 - 56)	52 (43 - 59)	40 (33 - 48)

mL/min, 1.73m^2

Figure 7.

*Percent changes of iohexol clearance from baseline. Medians, 25th and 75th percentiles. The inserted table gives medians, 25th and 75th percentiles of iohexol clearance. P values are denoted ** ($p < 0.01$) for differences between the groups, and †† ($p < 0.01$) for differences from baseline within the same group.*

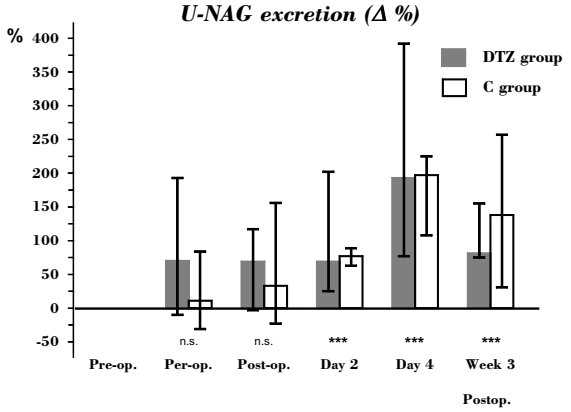


Figure 8.

*Percent changes of urinary N-acetyl-β-glucosamidase (U-NAG) levels from baseline in the patients who received diltiazem (filled bars) and controls (open bars). Medians, 25th and 75th percentiles. No significant differences were found between the groups. P-values are denoted *** ($p < 0.001$) for differences from baseline.*

DISCUSSION

After cardiothoracic surgery the incidence of PRD was found to be 11 %. It was also found that the mortality rate in PRD patients was 12 % as compared with 0.4 % in non-PRD patients. In cardiac surgery patients who received a perioperative infusion of the calcium channel blocker diltiazem, the postoperative glomerular function was better than in the control group. Postoperatively instituted ANP treatment improved GFR, natriuresis, and urine flow in patients after cardiovascular surgery. The potent vasodilator CGRP was shown to improve renal outcome in an experimental rat model of renal ischemia.

Postoperative Renal Dysfunction, PRD (Paper I)

Currently there is no universally accepted definition of criteria for renal dysfunction in connection with major surgical procedures, as also pointed out by Novis [Novis 1994] amongst others. This jeopardizes attempts to compare various renal outcome studies. In order to illustrate this the criteria for postoperative renal dysfunction used in other studies (see Appendix) were applied to the current patient population. This analysis is presented in **Table 4**. It was noteworthy to find that the incidence of PRD in the present

Table 4. The incidence of PRD including dialysis in a material of 1 030 patients undergoing cardiothoracic surgery utilizing cardiopulmonary bypass as described in Paper I when different published criteria for PRD are applied.

	Criteria for postoperative renal dysfunction incl. dialysis according to:			
	Mangano 1998	*Andersson 1993*	*Zanardo 1994*	*Paper I*
CABG	4.8 %	8.6 %	12.1 %	9.1 %
CABG + VR	13.0 %	15.6 %	24.5 %	20.7 %
VR	4.9 %	9.7 %	10.2 %	10.8 %
AA	11.5 %	27.6 %	20.0 %	24.2 %
Other	4.3 %	4.2 %	4.3 %	4.0 %
All patients	5.9 %	10.1 %	13.1 %	11.0 %

CABG = coronary artery bypass grafting, VR = valve replacement, AA = aortic aneurysm repair.

series was reduced from 9.1 % to 4.8 % when the criteria from the Mangano study [Mangano 1998] were applied to patients subjected to solitary CABG surgery in our material. Similarly, in the more complex group of combined CABG and valve replacements, corresponding incidences were 20.7 % and 13.0 %, respectively (**Table 4**).

These comparisons indicate that no interpretation of renal outcome studies can be carried out without simultaneously scrutinizing the criteria used for classification of renal dysfunction. It appears that the criteria for renal dysfunction set in the present study were not as limited as in some other studies, thus explaining the somewhat higher incidences of PRD. It is also of significant interest to compare the renal outcome in different studies when identical criteria are being used. This is shown in **Table 5** from which it can be deduced that comparable renal outcome was found in all four studies. Thus, there are two important issues observed. *Firstly*, postoperative renal dysfunction after major surgery is a significant clinical challenge that has to be addressed by studies on renal protection. *Secondly*, there is a need for an international consensus on criteria for postoperative renal dysfunction.

Renal Vasodilation 1 – CGRP (Paper II)

Although CGRP is well described as a most potent vasodilator its basic working mechanism has not yet been completely delineated [Villarreal 1994]. It is known that CGRP activates adenylyl cyclase *via* cell membrane bound G proteins and that the increased cAMP production *via* protein kinases activates ATP sensitive potassium channels [Siegel 1999]. This results in a hyperpolarization of the cell membrane which may affect voltage gated calcium channels resulting in relaxation of smooth muscle cells. In higher doses CGRP administration results in systemic hypotension as

Table 5. Comparisons of the incidences of PRD including dialysis in other patient materials *vs.* Paper I, when applying the respective published criteria for PRD.

PRD Criteria:	*Mangano1998*		*Andersson 1993*		*Zanardo 1994*	
Patient material:	Mangano	Paper I	Andersson	Paper I	Zanardo	Paper I
CABG	6.8 %	4.8 %	16.4 %	8.6 %	15.4 %	12.1 %
CABG + VR	15.1 %	13.0 %		15.6 %		24.5 %
VR					12.4 %	10.2 %
AA					36.1 %	20.0 %

CABG = coronary artery bypass grafting, VR = valve replacement, AA = aortic aneurysm repair

demonstrated in paper II. The 25 pmol/kg/min dose of CGRP reduced, in accordance with previous reports using high doses of CGRP [Villarreal 1988a], systemic blood pressures during and after renal ischemia. In the animals that received the lower CGRP dose of 10 pmol/kg/min, as well as in the control animals, reductions of systemic blood pressures during and after ischemia were less pronounced (**Figure 5**). This CGRP dose of 10 pmol/kg/min showed a renal protective potential. In the higher dose of 25 pmol/kg/min the systemic hypotension activates renin production [Skinner 1964] which enhances plasma levels of the vasoconstrictor angiotensin II. Theoretically, the resulting renal vasoconstriction may to some extent be counterbalanced by CGRP thus limiting the renin – angiotensin II effect initiated by the systemic hypotension. A CGRP effect that is mediated *via* pathways that are not dependent on ATP sensitive potassium channels [Reslerova 1998].

In paper II the plasma urea concentrations until day 7 after renal ischemia were, in the higher CGRP dose group as well as in controls, higher than baseline values. In the lower CGRP dose group, however, plasma urea levels were similar to baseline values already from day 3 after renal ischemia which demonstrates a dose related renal protective effect of CGRP. This relation appears to be due to that the lower CGRP dose avoids systemic hypotension and hence hypotension-induced stimulation of renin and angiotensin II production. In order to explore other possible explanations for the dose relation, *e.g.* receptor occupancy, a classical dose response study would have to be performed.
Based on the results in paper II, CGRP was found to be a promising agent for renal protection. Its renal protective capacity ought to be elucidated in future clinical investigations, to titrate a dose adequate for a positive effect on renal function, while systemic hypotension is avoided.

Renal Vasodilation 2 – ANP (Papers III and IV)

Normal renal function is important for the maintenance of body fluid and electrolyte balance. A prominent regulatory function for the kidney to achieve this rests on the effects of Atrial Natriuretic Peptide, ANP, secreted mainly from the atrial walls of the heart. The renal effects of the peptide are well described and consists of both vasodilatory and osmotic actions [Ballerman 1992]. Both papers III and IV supported these earlier findings of ANP actions on renal function and it was shown that urine flow, inulin clearance for GFR, and fractional sodium excretion were improved after major abdominal surgery (paper III) as well as after coronary revascularization (paper IV). These effects were reached at an ANP infusion rate (7.5 pmol/kg/min) that did not cause systemic hypotension. This speaks in favor of the thesis that ANP has a renal protective capacity. An interesting mechanistic feature of ANP is that it acts on the glomerular circulation as a vasodilator

on afferent and as a vasoconstrictor on efferent vessels [Ballerman 1992]. Consequently, ANP has the potential to directly affect glomerular hydrostatic forces as schematically illustrated in **Figure 9**. ANP may also interact with the mesangium to increase the area of filtration [Lakkis 1997] further enhancing GFR. In addition, ANP´s vasodilating effects on the vascular smooth muscle has been shown to counteract the effects of potent renal vasoconstrictors such as norepinephrine and angiotensin II [Laragh 1988] and it also decreases renin as well as vasopressin release [Laragh 1988, Fujio 1986]. Hence, ANP not only acts on renal function *via* vasodilation but also through a unique differentiated effect on afferent and efferent glomerular vessels, with the possibility for a combined treatment strategy with agents working through other mechanisms, to reach additive renal protection effects.

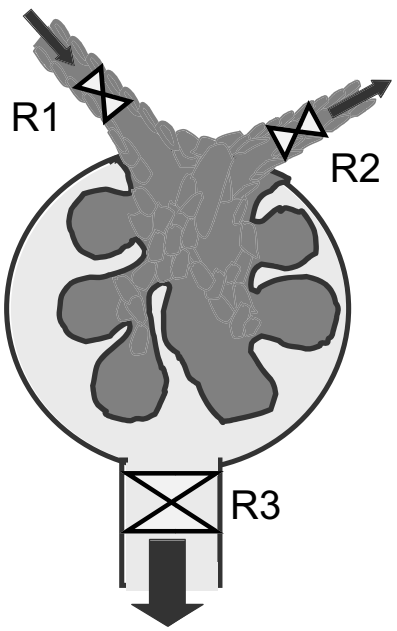


Figure 9. *The glomerulus. The serial resistors R1 and R2 determine RBF, since R3 >> R1 and R2. GFR is determined by the changes of R1, R2 and R3 in different combinations. Adapted from Greger [Greger 1996].*

Renal Vasodilation 3 – Diltiazem (Papers V and VI)

Since most treatment strategies for renal protection are directed towards improved renal perfusion, they may, as discussed above, cause unwanted systemic hypotension. In relation to this it was noteworthy, that the bolus dose and infusion rate of the calcium channel blocker diltiazem in paper V, did not cause hypotension. On the basis of this information the diltiazem dose for paper VI could be estimated. This resulted in a slightly lower bolus dose (0.28 mg/kg in paper V and 0.25 mg/kg in paper VI) whereas the infusion rate was increased from 1.0 µg/kg/min in paper V to 1.7 µg/kg/min in paper VI. These doses were comparable with those used in other investigations [Colson 1992, Seitelberger 1994] and the dose adjustments were implemented without reduction of systemic blood pressures.

The choice of a calcium channel blocker for renal protection after major surgery is a logical one, since the influence on cytosolic calcium concentrations in vascular smooth muscle cells is directly related to the smooth muscle tone. In fact, the ultimate common signal transduction

pathway for most vasoregulators is to modify intracellular calcium concentrations [Siegel 1996]. As a reflection of this, several investigations of renal function and calcium channel blockers have been performed [Lumlertgul 1991, Epstein 1992a, Amano 1995]. In general, a beneficial effect has been found. In conformity with these studies, the current results (paper VI) also demonstrated a positive effect on renal function. This outcome was attained in patients who had a known preoperative renal dysfunction as evidenced by serum creatinine values of > 120 and < 300 μmol/L. Renal function in diltiazem treated patients was preserved postoperatively, and even improved three weeks after cardiac surgery, as measured by iohexol clearance ($p < 0.01$, **Figure 7**). Controls, however, showed a reduction of GFR (iohexol clearance) both in regard to baseline values ($p < 0.01$) and compared to the diltiazem group ($p < 0.01$). Based on these findings, it can be stated that calcium channel blockers do indeed protect renal function in connection with cardiac surgery.

Future Aspects

The incidence of renal dysfunction after cardiovascular surgery is still high and calls for renal protective measures. In the current series of investigations, three different, potentially nephroprotective agents, CGRP, ANP, and the calcium channel blocker diltiazem, were the objects of study. It was shown that diltiazem, in patients with preexisting renal impairment, protected and even improved their renal function after cardiac surgery. In future studies it would be of interest to further explore the renal outcome after major surgery, trauma and shock, using as protective agents both CGRP and ANP, not only separately but also in combinations including diltiazem.

Conclusions

Renal impairment after major surgery is still a significant clinical entity, closely associated with other complications and increased mortality. It was concluded that:

* the incidence of postoperative renal dysfunction (PRD) after open heart surgery was 11 % (**I**)

* the mortality rate in PRD patients was 12.4 % compared with 0.4 % in those who did not develop PRD (**I**)

* the potent vasodilator CGRP had a protective effect on renal function after experimental renal ischemia in rats. The protective effect was dose related as higher CGRP doses resulted in systemic hypotension (**II**)

* the glomerular filtration rate (GFR), urine flow, and sodium excretion improved after ANP infusions in humans on the first postoperative day after abdominal aortic surgery (**III**)

* also in the immediate postoperative period ANP infusions improved renal function after open heart surgery (**IV**)

* there were no hemodynamic side effects from diltiazem treatment, neither with a bolus dose of 0.28 mg/kg followed by an infusion of 1 μg/kg/min (**V**) nor with a bolus dose of 0.25 mg/kg followed by an infusion of 1.7 μg/kg/min (**VI**)

* the calcium blocker diltiazem prevented from a further worsening of the preexisting renal dysfunction after open heart surgery. Indeed, glomerular filtration rates were improved three weeks postoperatively in patients treated with diltiazem (**VI**).

APPENDIX

PRD Criteria

Mangano 1998 excluded patients with preoperative renal failure or dysfunction (S_{Cr} > 177 µmol/L), and cases where perioperative S_{Cr} were not available, including early mortality. Postoperative renal failure in the remaining patients were defined by the need for new dialysis after surgery, and postoperative renal dysfunction as a postoperative S_{Cr} level of 177 µmol/L or greater and an increase of 62 µmol/L or greater from preoperative to maximum postoperative values.

Andersson 1993 excluded cases where perioperative S_{Cr} were not available and defined postoperative ARF as patients receiving dialysis *de novo* after surgery, and patients with a peak S_{Cr} level at least 50 % higher than the preoperative value.

Zanardo 1994 evaluated only patients with preoperative S_{Cr} < 133 µmol/L (= 1.5 mg/dL). Renal dysfunction was defined as a maximum postoperative creatinine level of 133 to 221 µmol/L, and acute renal failure as peak S_{Cr} > 221 µmol/L.

In **Paper I** patients with preoperative dialysis were excluded, as well as cases where perioperative S_{Cr} were not available, including early mortality. Postoperative renal dysfunction was defined as postoperative dialysis *de novo*, a GFR reduction ≥ 33% (equivalent to a 50% S_{Cr} increase), and/or a GFR reduction ≥ 25% if a lowest GFR < 30 mL/min/1.73m^2 was noted.

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