PHARMACOLOGICAL MANIPULATION OF GLOMERULAR BARRIER FUNCTION

FARMACOLOGISCHE BEÏNVLOEDING VAN DE GLOMERULAIRE BARRIÈRE

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Contents

Chapter 1

General introduction

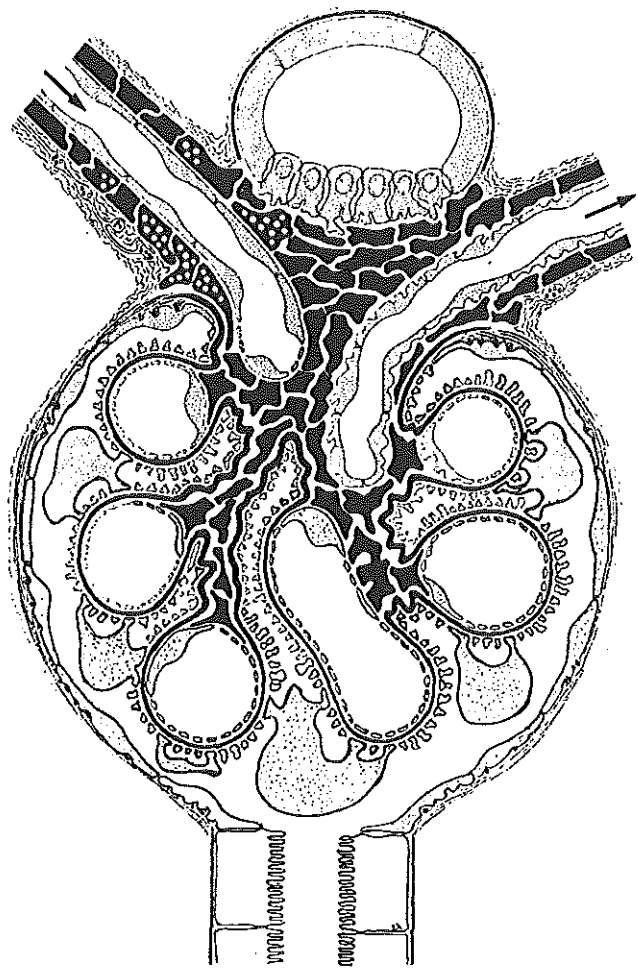
1.1 Structure of the glomerular filtration barrier.

The formation of urine is the result of two opposing processes. A highly dilute pro-urine is produced by ultrafiltration across glomerular capillaries. Extensive reabsorption in the tubules reduces the 180 litres of plasma that are filtered daily to approximately 2 litres of urine which are excreted under normal conditions. The driving force of this massive filtration is a net pressure gradient (hydrostatic pressure difference minus the oncotic pressure of plasma) of only 10 mmHg. The glomerular filtration barrier has the remarkable ability to allow the passage of these vast amounts of fluid while almost totally excluding proteins from the urinary compartment, despite the high concentration of macromolecules in human plasma. The glomerulus therefore acts as a very efficient sieve. The glomerular capillary wall consists of several layers with varying contributions to the sieving characteristics of the kidney (Fig. 1).

The glomerular capillaries are lined with endothelial cells. This cell-layer is perforated by fenestrae, with an average diameter of 50 to 100 nm, which compose about 13 percent of the capillary surface area [1]. Such fenestrae are characteristic of capillaries with high hydraulic conductivity. Due to the large size of the fenestrae, little restriction in the flux of not only water but also

macromolecules would be expected to occur at the level of the endothelium. However, as the endothelial surface is coated with negatively charged glycoproteins which repel negatively charged proteins, a certain degree of charge selectivity may be exerted by the endothelial layer of the filtration barrier [2]. After passing through the fenestrae, the next impediment to the transcapillary passage of macromolecules is the glomerular basement membrane (GBM), which covers the capillary loops except for the axial region of the capillary tuft where it borders the mesangium [3]. The GBM is directly exposed to plasma proteins because of the fenestrations within the endothelial layer. It is also in direct contact with the mesangium, which is a basement membrane-like intraglomerular matrix produced by the mesangial cells [4]. Mesangial cells contain bundles of densely interwoven microfibrils. These actin containing bundles are tightly connected with the GBM and are capable of contraction [5]. Mesangial contraction is thought to modulate the tension of the glomerular basement membrane and could thereby regulate the filtration surface area [6]. In contrast to most basement membranes, the GBM is a fusion product of two basement membranes produced by endothelial and epithelial cells (podocytes) [7]. This special construction is responsible for the typical laminated structure of the GBM which possesses a rather thick, duplicated lamina densa sandwiched between two external laminae rara. The central electron dense layer (lamina densa) consists of a compact meshwork of type IV collagen fibres [8][9]. The lamina densa has long been considered to be the only determinant of glomerular size-selectivity, although more recently other constituents of the capillary wall have been shown to contribute to its size-selective properties [10]. The "backbone" of collagen fibres lends the GBM its characteristic mechanical stability [11]. Other components of the basement membrane are attached to this three dimensional network. The collagen fibres are surrounded by laminin [12], and are further associated with heparan sulphate proteoglycans, which are especially prominent in the laminae rara [13,14]. As was initially demonstrated by electron microscopy using cationic probes, the high content in anionic sulphate residues results in a negative charge which [15]. One function of the proteoglycans could be the charge selective control of filtration through the GBM. Furthermore, some proteoglycans may be important in the continuous process of formation and degradation of the basement membrane, either through control of serine protease

Fig. 1



Longitudinal section through a glomerulus. The capillary network together with the mesangium is enclosed in a common compartment bound by the GBM. The outer aspect of the GBM is covered with epithelial cells (podocytes), whereas the inner surface of the capillaries is lined with fenestrated endothelial cells [7].

activity [16], or as a result of the action of fibroblast growth factor [17] and TGF-β [18]. Recent studies with electron microscopy of tissues processed after rapid-freeze substitution show that the separation of the GBM in three distinct layers might be an artifact and that the GBM may have a uniform appearance throughout its entire thickness [19].

The final obstacle to the transglomerular passage of proteins consists of a layer

of epithelial cells. Epithelial cells exhibit highly differentiated foot-processes with a well-developed contractile apparatus [20]. These podocytes are connected to the basement membrane through β_1-integrins [21], proteoglycans [22], as well as other macromolecules [23]. The attachment of podocytes to the GBM and to each other through the slit pore membranes allow them to withstand the relatively high (40 mmHg) hydrostatic pressure difference between the capillary and Bowman's space [24]. Treatment with antibodies directed at β_1-integrins leads to epithelial cell detachment from the GBM and marked proteinuria [25]. Neutralisation of anionic basement membrane molecules also results in proteinuria through detachment and simplification of the foot processes [26]. Foot processes interdigitate with those of adjacent cells. The foot processes are adjoined to the neighbouring cell by slit-pores, a unique type of cell junction. These slit-pores cover from 3 to 10% of the GBM area [27]. At very high magnification a mesh-like structure of the slit-pore membrane has been observed [10]. The size of the resulting pores was estimated to be approximately 38 Å [28].
Thus the barrier to the glomerular filtration of macromolecules consists of a course filter (the GBM) followed by a fine filter (the filtration-slit membranes).

1.2 Glomerular filtration of macromolecules.

Concentrations in Bowman's space of substances with a molecular radius the size of inulin and smaller ($<$ 16 Å) are the same as those in plasma. In contrast, under normal conditions only small amounts of proteins, of the size of albumin and larger, are found in the ultrafiltrate. The Bowman's space to plasma ratio of a given macromolecule, known as the sieving coefficient (θ), is a measure of the permeability of the filtration barrier to the macromolecule. In humans the only approximate measure of the sieving coefficient of a macromolecule is its so called fractional clearance, which is the clearance of the macromolecule divided by the clearance of a marker such as inulin that passes the glomerular capillary wall freely. By comparing fractional clearances of different macromolecules with their plasma to Bowman's space ratios obtained by micropuncture, it was found that the restriction of macromolecules is directly in proportion to their size [29][30].

Conflicting data exist concerning the exact location size and charge selectivity within the glomerular filtration barrier. In situ dripfixation using gold-stained proteins reveals that neither size nor charge selectivity is an all or nothing phenomenon [31]. Concentrations of albumin increase gradually towards the outer side of the GBM. Daniels et al. were able to consolidate rat GBM into a homogeneous layer atop a polysulphone filter [32]. They observed that both hydraulic and macromolecular permeability of the denuded GBM are fifty times greater than that of the intact glomerulus. This suggests a major contribution of cellular elements to glomerular permeability properties. The importance of the glomerular cell layer was further emphasised by the observation that the frequency of filtration slits is a more important determinant of glomerular hydraulic permeability than the increase in average path length for the filtrate due to increased membrane thickness [33].

A major limitation of the use of plasma proteins to probe glomerular barrier function is their tubular reabsorption. Normally, most filtered protein is reabsorbed in the proximal tubules, a process which is also charge dependent and varies over time [34]. For this reason differential solute clearance techniques which employ various exogenous test polymers as test solutes have been developed [35,36]. The substance most often used in humans is neutral dextran [37,38]. As dextran is neither reabsorbed nor secreted by tubular cells, its fractional clearance equals the glomerular sieving coefficient (concentration in plasma/concentration in Bowmans space). Usually a solution containing dextrans of broad size distribution is infused and the renal clearances of numerous fractions with different radii are measured. These clearances are corrected for the transglomerular water flux (GFR). Plotting the sieving coefficients of the dextran fractions against their stokes radius (Å) yields a so called sieving curve.

In normals the sieving coefficients for dextrans are unexpectedly large given the absence of proteinuria [39]. This is probably due to the fact that dextran is a flexible linear polymer of glucopyranose which differs in configuration from globular proteins [40]. Oliver et al. developed a model for glomerular filtration of random-coil macromolecules like dextrans [41]. Unfortunately, the results of these calculations are influenced by the unknown magnitude of attractive interactions between macromolecules and pores which are dependent on solute concentration [42]. Also, the effect of branching in dextran molecules may not

be negligible, despite the fact that branches are relatively short and infrequent [43]. Therefore Ficoll is used in recent animal studies, a cross-linked copolymer of sucrose and epichlorohydrin which behaves more like an ideal spherical molecule [44]. Using Ficoll, Oliver et al. were able to show that the actual sieving coefficients of the glomerular barrier may be ten-fold lower [45]. Unfortunately humans lack the enzyme to degrade Ficoll, which precludes its use in human studies.

Anionic proteins are more restricted then cationic proteins of equivalent size. Albumin has a hydrodynamic diameter of 36 Å, which is considerably smaller than the glomerular pore-size which is approximately 55 Å in width when estimated using dextrans [46]. Recent data obtained using fractional Ficoll clearances indicate that the effective pore radius may be as small as 29 Å which would mean that size-selective has a marked influence on the sieving of albumin [45]. However, the filtrate to plasma concentration ratio of Ficoll would still be 30 times greater than that of albumin. Therefore the restriction of albumin to the plasma compartment can only be explained by assuming that the glomerular barrier also discriminates on the basis of charge. Sulfated proteoglycans are abundant in the GBM and on the surface of epithelial cells [13]. This results in a negative charge of the glomerular barrier. Because of this, albumin, which is also negatively charged at physiological pH, does not easily pass the GBM. However, charge selectivity was not altered in isolated GBM treated with heparinase and glomerular cells may be of critical importance in the regulation of glomerular charge selectivity [47]. The charge selective properties of the glomerular barrier were demonstrated using negatively charged dextrans (dextran sulfate), in animals and, more recently, also in humans [48-50]. Unfortunately dextran sulfate binds to serum proteins. This binding is size-dependent which makes the accurate calculation of fractional clearances difficult [49]. Furthermore, the adminstration of dextran sulfate is potentially hazardous. Therefore, in order to study charge selectivity, investigators can only use fractional clearances of pairs of differently charged endogenous proteins identical in size, such as albumin versus glycated albumin, IgG versus IgG_4 or amylase isoenzymes [51-54]. As mentioned above the charge selective nature of tubular reabsorption must be taken into account when interpreting the results obtained with these endogenous proteins.

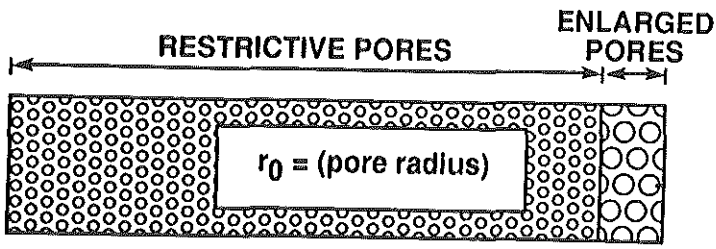
1.3 Mathematical modelling of the filtration barrier.

The transglomerular sieving of uncharged macromolecules is not solely determined by the size selective properties of the glomerular barrier [55]. Changes in the determinants of glomerular filtration have marked effects on the sieving of dextrans. Increases in the ultrafiltration coefficient K_f (hydraulic permeability x surface area) and plasma oncotic pressure both lead to increased fractional clearances of neutral dextran molecules. On the other hand, increases in glomerular plasma flow and the transcapillary hydraulic pressure gradient ΔP both lead to decrements in the fractional clearance of dextrans [56], although an increase in ΔP may result in secondary changes in size-selectivity [55,57]. Using mathematical models, the observed dextran sieving behaviour can be corrected for (changes in) the determinants of GFR.

These models have two principal features. Firstly, they are based on a description of the intrinsic barrier properties of the glomerular capillary wall. Secondly, they make use of a mass balance equation that relates changes in concentration of the test solute along the glomerular capillary to the rate at which that solute is filtered. Deen et al [36] compared several mathematical models of glomerular barrier function based on the hydrodynamic theory for hindered transport of rigid, spherical, uncharged macromolecules [58]. In these models, these capillaries are supposed to be permeated by a number of cylindrical pores. The glomerulus is represented as a number of parallel capillaries of identical diameter and length. This approach was proved to be valid because the calculated membrane-pore parameters were relatively insensitive to differences in assumed distribution of values of capillary length [39,59,60]. Also, the failure of these calculations to consider the pulsatile nature of glomerular perfusion induced negligible errors in calculating membrane parameters from experimental data [61,62].

When the distribution in diameter of these pores was considered to be uniform (isoporous model) the characteristic biphasic appearance of the empirical sieving curves could not be adequately be explained. Therefore heteroporous models were introduced which assume two differently sized pores, or pore-sizes with normal or log-normal distribution [63]. The model that predicted the experimental data most accurately was one in which the GBM was depicted as a filter in which

Fig. 2



"Isoporous + shunt" model of the glomerular filtration barrier as developed by Deen et al. [36] in which most pores are supposed to have an identical radius whereas a small subpopulation of pores are large and not size-restrictive.

most pores are of identical radius r_0 and that has a small number of large pores, which do not discriminate on the basis of size (isoporous + shunt model) (Fig. 2) [36]. The fraction of filtrate passing through these nonrestrictive pores is represented by ω_0. The intrinsic permeability characteristics of the GBM are fully described by K_f, r_0, and ω_0. Curve fitting techniques allow for the approximation of these variables.

An important assumption in these calculations is the value of ΔP, which cannot be measured directly in humans, and which correlates inversely with the ultrafiltration coefficient K_f [64]. Recent efforts to obtain an accurate value of ΔP have proven unsuccessful [65]. Daniels et al. developed a method using intact glomeruli which allows the approximation of K_f in vitro [33]. These measurements allow for direct calculation of ΔP. This technique was used to measure the determinants of glomerular hypofiltration shortly after renal transplantation [66]. Unfortunately, the necessity of obtaining renal tissue by biopsy limits the use of this technique in clinical practice. Lacking such data, the results of calculations of K_f, r_0, and ω_0 should be viewed as semi-quantitative approximations only. Still, valuable information can be obtained as to the direction of changes. At present calculations using mathematical models of the

glomerular barrier are the only tools in the effort to correct changes in dextran sieving for changes in renal hemodynamics that occur simultaneously.

1.4 Glomerular barrier function in human renal disease.

Minimal change disease.

In minimal change disease (MCD) histological alterations are limited to broadening and simplification of the glomerular epithelial foot processes [67]. In this disorder the increase in albumin excretion is more prominent than that of larger proteins such as immunoglobulins. Clinically this results in a high selectivity index (the quotient of IgG and albumin clearances). GFR and renal plasma flow are usually well preserved in patients with MCD [68]. When compared to healthy controls, the sieving of small dextrans < 50 Å is reduced. Sieving coefficients of larger dextrans are similar to corresponding values in controls. When applying the mathematical model described above to these findings, they fit best with the assumption that the ultrafiltration coefficient K_f is markedly decreased, while the flow through the non-discriminatory shunts is slightly increased [69]. This implies a significant decrease in the number of pores available for filtration with only minor changes in size-selectivity. The change in calculated permselectivity was not of a sufficient magnitude to explain the massive increase in albumin excretion observed in these patients [68]. Therefore a second defect must exist in glomerular permeability to proteins. Such a defect is likely to be the result of impaired charge selectivity. Reduction of the charge-selective properties of the GBM has been well documented in patients with MCD [70,71] as well as in animal models of MCD [72]. Using a model for the glomerular filtration of charged solutes [73] the degree of albuminuria in MCD was attributable to a 50% reduction in the concentration of fixed negative charges in the glomerular capillary wall [74].

Focal segmental glomerular sclerosis

In focal segmental glomerular sclerosis (FSGS) the glomerular passage of small

dextrans is impaired to a similar extent as in MCD. Renal plasma flow is usually lower in FSGS [75,76]. Because of this difference in flow one would predict the fractional excretion of dextrans to be higher in FSGS [56]. In order to explain the absence of a difference in sieving of small dextrans between MCD and FSGS, we have to assume that the K_f in FSGS is substantially lower than in MCD. Calculations based on the "isoporous + shunt" model infer that this is the consequence of a reduced total number of restrictive pores of the major isoporous component of the membrane [77]. In FSGS the sieving of large dextrans (> 54 Å) is increased. This suggests that the GBM is less size-selective. The calculated "shunt-flow" in FSGS was approximately twice that in MCD. As in MCD, the proteinuria observed in patients with FSGS cannot be fully accounted for by the magnitude of the increase in flow through the non-selective shunts. Therefore a decrease in glomerular charge selectivity must contribute to the proteinuria in patients with FSGS, although to a lesser extent than in MCD.

Membranous glomerulopathy

In membranous glomerulopathy (MG) the fractional clearance of large dextrans exceeds that in FSGS. The calculated membrane parameters K_f, r_0 and ω_0 point towards a more severe disruption of the size selective properties in the glomerular barrier. In contrast to the previously discussed disease states, in MG theoretical albumin and IgG clearances through the shunt pathway could account for the observed proteinuria [78]. The size-selective defect in MG correlated with the amount of histological changes in the capillaries [79]. K_f calculated in MG from dextran sieving behaviour, is even lower than in FSGS. The resistance to water flow due to both a three-fold widening of the basement membrane, and a reduced filtration slit frequency are the most likely causes of the progressive renal insufficiency in MG [80-82].

IgA nephropathy

In the early stages of this disease a diminished charge selective barrier has been suggested using cationic probes [83]. Using dextran sieving techniques a disruption of the size-selectivity of the glomerular wall could be demonstrated as

well [84]. The degree in which glomerular size-selectivity is impaired correlates with the mesangial sclerosis index, which is a histological measure of the severity of the disease [85].

Diabetic nephropathy

When the proteinuria in patients with diabetic nephropathy can be demonstrated using routine urine analysis (dipstick) protein excretion rate exceeds 200 mg/day, and these patients are considered to have "macroproteinuria" [86]. Various reports have demonstrated that in diabetic subjects proteinuria of this magnitude is accompanied by abnormal dextran sieving behaviour [87-89]. In addition, more recently, alterations in the permeability to dextrans have been observed in the early phase of diabetic nephropathy (micro-albuminuria) [90]. The sieving curve of uncharged dextrans shows a slight, but significant, decrease in the clearance of small dextrans pointing towards decreased K_f. The difference in shunt flow (ω_0) between IDDM and healthy subjects was not significant. With advancing diabetic nephropathy, as indicated by increased proteinuria and decreasing GFR, the alteration of K_f becomes more apparent [91-93]. Permselectivity is further reduced in these later stages since larger dextrans pass the GBM more freely.
In most studies in diabetic nephropathy the proteinuria exceeds the predicted passage of proteins through the shunts [51,88]. Decreased charge selectivity is supposed to contribute to the albuminuria especially in early phases of diabetic nephropathy. Diabetic nephropathy appears to consist of a continuum of progressively deranged glomerular capillary wall function. This derangement is characterised by a progressive reduction in the number of glomerular pores accompanied by a shift in the pore-size toward a larger radius, i.e. increase in ω_0. The disproportionate increase of the clearance of albumin compared to other proteins could be the result of impaired electrostatic retardation. A decrease in heparan sulphate, the major constituent of GBM negative charge, has been demonstrated in humans [94-98] as well as animal models of diabetic nephropathy [99], and this decrease correlates with the magnitude of the increase in permeability [100]. As the disease progresses altered cross linking may occur through a further alteration in GBM composition which leads to a further decreased in size selectivity [101,102]. The potential importance of the negatively

charged constituents of the GBM in maintaining size-selectivity was demonstrated by selective depletion of GBM negative charge using a polycation which resulted in marked decrease in size-selectivity [103]. Thus distortion of GBM gel structure may lead to altered porosity.

1.5 Effects of physiological and pharmacological interventions on glomerular barrier function.

Volume expansion

In nephrotic subjects, volume expansion with colloid solutes increases proteinuria and dextran clearances. This is likely to be caused by increased shunt flow [104]. In normal subjects volume expansion does not alter urinary protein excretion; clearance studies suggest a slight decrease in K_f only [105].

Changes in protein load

In normal subjects and patients with nephrotic syndrome, oral protein loading leads to a significant increase in the urinary excretion and fractional clearance of IgG as well as albumin [106,107]. This suggests that decreased size-selectivity is decreased in these patients. In accordance with these findings is the fact that low protein diet ameliorates the defect in size-selectivity observed in rats with reduced renal mass [108,109]. This effect may be secondary to a reduction of angiotensin II [110]. Some studies in humans have also shown amelioration of the size-selective defects after protein restriction [111,112]. In patients with membranous nephropathy however, a low-protein diet did not improve permselectivity [113].

Diuretics

The administration of furosemide increases protein excretion in patients with moderate proteinuria [114]. This proteinuric effect is the result of increased ω_0, i.e. decreased size-selectivity. The increased passage of proteins correlated well

with the observed increases in GFR and filtration fraction. The increase in proteinuria after the administration of furosemide is accompanied by an increased excretion of prostanoid metabolites [115]. Also, the effects of furosemide are abolished by pretreatment with indomethacin [116]. These findings could indicate involvement of renal prostaglandins.

Prostaglandins

Prostaglandins are capable of antagonizing vasoconstriction and glomerular contraction induced by angiotensin II [117]. They are believed to play a critical role in the maintenance of glomerular filtration under conditions of reduced renal perfusion [118]. Both infusion of PGI_2 and administration of misoprostol, a stable PGE_1 analogue, lower the fractional clearances of dextrans of 40 Å or less, despite opposite effects on renal plasma flow [119]. Non steroid anti-inflammatory drugs such as indomethacin are well known to reduce protein excretion in the clinical setting [120,121]. In membranous nephropathy, a selective reduction in the clearance of larger dextrans has been demonstrated following the administration of indomethacin, associated with a corresponding reduction in filtrate traversing the shunt pathway [122]. These changes in size-selectivity are closely associated with a reduction in GFR, suggesting that the capacity to alter proteinuria is linked with changes in glomerular hemodynamics which are known to induce reversible changes in glomerular permeability [123,124].

Renin-angiotensin system

Infusion of renin was shown to induce proteinuria in the rat as early as 1949 [125]. More recently, a similar increase in proteinuria was observed following the administration of angiotensin II [110]. Systemic infusion of angiotensin II predominantly constricts the efferent arteriole with increased glomerular capillary pressure and decreased glomerular plasma flow as a result [126]. In addition, through constriction of mesangial cells and subsequent reduction of the filtration surface area, angiotensin II reduces K_f. In rats infusion of angiotensin II induces a marked increase in glomerular permeability and a loss of the size-selectivity of

the glomerular barrier [110]. The increased filtration fraction and net perfusion pressure are thought to contribute to this alteration in permselectivity.
Data in humans concerning the infusion of angiotensin II are conflicting with those reported in rats. No changes in permselectivity could be demonstrated in patients with nephrotic syndrome both when angiotensin II was used in a pressor dose [127], or in sub-pressor doses [128]. In fact, the excretion rates of both albumin and IgG fell significantly during A II infusion, most likely as a result of reduced filtered protein load [127]. Clinically the use of angiotensin converting enzyme (ACE) inhibitors has been shown to be effective in the reduction of proteinuria [129]. Studies with graded dextrans demonstrated a reduction in shunt flow after ACE inhibition, indicating increased permselectivity [130]. Similar data have been obtained using an angiotensin II AT1 receptor antagonist, suggesting that the increase in permselectivity during ACE inhibition is indeed the result of reduced formation of angiotensin II [131-134]. The specific advantage of ACE inhibition over other methods of blood pressure reduction is still disputed [135]. Recent data suggest that beside the hemodynamic alterations that contribute to a reduction in proteinuria, ACE inhibitors, when administered over a longer period of time, may structurally alter basement membrane permeability [136,137].

Other

Recently, attention has been focused on the effects of biological mediators of inflammation on the glomerular barrier function. Platelet activating factor (PAF) induces an increase in protein excretion in the Sprague-Dawley rats without evident changes in renal hemodynamics [138,139]. The passage of large dextrans through the GBM was selectively increased through an increase in shunt-flow. This effect appears to be independent of changes in cyclo-oxygenase products since a specific inhibitors did not prevent this phenomenon [140].
In MCD a circulating cationic protein, vascular permeability factor (VPF), has been implicated in reducing glomerular negative surface charge [135]. VPF might be an interleukin or highly related substance [141]. The reduction in negative surface charge in MCD may account for the decrease in charge selectivity.

1.6 Aims and scope of the thesis.

The aim of the studies presented in this thesis was to gain more knowledge concerning the way in which various diseases affect glomerular barrier function. To do so we measured fractional clearances of both uncharged dextrans and endogenous proteins to probe the glomerular filtration barrier. We also studied the alterations in glomerular permeability caused by treatment with cyclosporine A, infusion of atrial natriuretic peptide or treatment with an ACE inhibitor, to assess the degree in which the glomerular permeability to macromolecules can be modified in various disease states associated with increased protein excretion. By measuring dextran clearances we hoped to differentiate between the hemodynamic determinants of GFR such as renal plasma flow on the one hand, and changes in the intrinsic permeability properties of the glomerular filtration barrier on the other, as mechanisms in the pathogenesis of proteinuria

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Optimising glomerular filtration rate and effective renal plasma flow measurements using a simple pharmacokinetic model

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Abstract

We applied an open one compartment pharmacokinetic model for the determination of glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) based on a rapid intravenous loading dose followed by a constant infusion of ^{125}I-iothalamate and ^{131}I-orthoiodohippurate in order to ensure constant plasma levels of the two clearance markers. The loading dose was based on the assumption that the volume of distribution of the two markers equals the extracellular volume (25 % of the body weight). The infusion rate was calculated after the clearance of thalamate was estimated from body weight, age, sex and serum creatinine using Cockcrofts formula. The clearance of hippurate was assumed to be four times that of thalamate.

We studied the reliability of this model in 212 patients with insulin dependent diabetes mellitus (IDDM; n=74), nephrotic syndrome (NS; n=18) and heart (HTX; n=69) or kidney (KTX; n=51) transplants.

A steady state concentration was obtained in all patient groups, even when GFR was markedly depressed. In patients with diabetes, we observed more variance between plasma and urinary clearances of thalamate, which could be due to inaccuracies in urine sampling. In these patients, GFR should be measured using a method that is not dependent on urine collection. Also, the estimation of GFR

by means of Cockcrofts equation seems to underestimate GFR in diabetic subjects.

Introduction

The clearance of inulin and para-aminohippurate have classically been assumed to be a measure for the glomerular filtration rate (GFR) and effective renal plasma flow (ERPF). The chemical analytical methods for measuring both inulin and para-aminohippurate are cumbersome [1]. The quantification can be simplified by using ^{125}I-iothalamate in stead of inulin, and ^{131}I-orthoiodohippurate in stead of para-aminohippurate [2]. The clearance of ^{125}I-iothalamate and ^{131}I-ortho-iodohippurate can be calculated by applying the one-compartment or two-compartment open pharmacokinetic models using either the single bolus injection technique or alternatively the constant infusion technique [3,1]. The rapid intravenous bolus technique requires 6 to 8 blood samples and exact timing of blood sampling. [4]. However, this pharmacokinetic model appears to be an oversimplification of the situation which may lead to underestimation of the renal clearance [5]. The continuous constant infusion technique with timed urine sampling for measuring the clearance of thalamate and hippurate avoids this problem. This technique requires water loading in order to ensure reliable urine collections.

It is possible to determine the clearance of thalamate or hippurate by dividing the infusion rate of the radio-labelled drugs by the plasma levels of these markers providing steady state plasma concentrations of the clearance markers. The advantage of this approach is that collection of urine is not necessary. However, steady state plasma concentrations are not readily achieved in patients with impaired renal function [6]. We have therefore developed a simple method for the simultaneous measurement of GFR and ERPF. The method is based on a loading dose immediately followed by a constant intravenous infusion of the radioactive compounds. Loading dose and infusion rates of the clearance markers are calculated after estimation GFR using Cockcrofts formula [7]. This method ensures steady state plasma concentrations of the radiopharmaceuticals within a

90 minute period, even in patients with severely depressed renal function.
We studied this method for the determination of GFR and ERPF in 212 consecutive patients with serum creatinine concentrations ranging from 52 to 728 μmol/L. In these patients, the renal and plasma iothalamate clearance, endogenous creatinine clearance and the estimation of GFR using Cockcrofts equation were compared.

Material and methods

Patients

We studied 212 consecutive patients referred to our department for the measurement of glomerular filtration rate (GFR) and effective renal plasma flow (ERPF). These included patients with insulin dependent diabetes mellitus with creatinine levels within the normal range and no overt macroalbuminuria and no medication except insulin (IDDM; n=74). Microalbuminuria (albumin excretion rate 30-300 mg/24 hr) was observed in 22 of these patients. We also studied patients with nephrotic syndrome (NS; n=18), heart transplant recipients (HTX; n=69) and children with kidney transplants (KTX; n=51). The clinical characteristics of the patients are shown in Table 1.

Table 1

Clinical patient characteristics

	n =	sex (m/f)	AGE years	S Creatinine μmol/L
Heart transplantation	69	53/16	46 (1.3)	181 (5)
Kidney transplantation	51	28/23	16 (0.5)	148 (19)
Diabetes mellitus	74	41/33	37 (1.4)	84 (2)
Nephrotic syndrome	18	7/11	45 (2.4)	140 (31)
Total	212	129/83	35 (1.1)	137 (6)

Methods

All patients received an oral water load of 300 ml after which diuresis was ensured by drinking 150 ml tap water each hour. One forearm vein was used for the infusion of radiolabelled clearance markers, while blood samples were drawn from the other arm. Patients remained in the supine position but were allowed to stand in order to pass urine. All measurements were performed between 8.00 and 12.30 AM.

Sustaining infusion

The solution for the continuous maintenance infusion contained 0.25 μCi/ml ^{125}I-iothalamate and 2 μCi/ml ^{131}I-orthoiodohippurate (Amersham, UK).

First an estimation of the thalamate clearance (CL_{est}Thal) was made using Cockcrofts equation [7]:

$$CL_{est}\,Thal\,(ml/min) = \frac{(140 - age) \times Body\ weight(kg)}{Serum\ creatinine(\mu mol/L) \times 0.815}$$

CL_{est}Thal was corrected for gender; for females CL_{est}Thal is 0.85 times that in males. Then the infusion rate (R, ml/min) was calculated from:

$$R\,(ml/min) = \frac{C_{ss}(cpm/ml) \times CL_{est}\,Thal\,(ml/min)}{Infusate\ concentration(cpm/ml)}$$

in which C_{ss} is plasma steady state concentration (cpm/ml). In each patient, the infusion rate was adjusted to yield plasma thalamate and hippurate concentrations of approximately 500 and 150 cpm/ml respectively. Depending on renal function, the infusion rate varied from 1 to 9 ml/hr.

Loading dose

The priming dose (D, ml) necessary to achieve the calculated steady state level C_{ss}, was calculated from:

$$D(ml) = \frac{C_{ss}(cpm/ml) \times V_d(ml)}{Infusate\ concentration(cpm/ml)}$$

where V_d is the volume of distribution for thalamate. We assumed this to be equal to the volume of the extracellular compartment, i.e. 0.25 x body weight. The clearance of hippurate was estimated to be four times that of thalamate. Therefore, in relation to the amount infused, the C_{ss} of hippurate will be only one fourth of that of thalamate. Thus, the required loading dose of hippurate need only be one fourth of that of thalamate. The loading dose contained 0.25 μCi/ml ^{125}I-thalamate and 0.5 μCi/ml ^{131}I-hippuran (one fourth of the hippurate concentration in the sustaining infusion). An example of the calculation of the intravenous loading doses of ^{125}I-thalamate and ^{131}I-hippuran and the infusion rate for the maintenance infusion of these compounds is given in Table 2.

Table 2

Example of the calculation of the loading doses and infusion rates

Age	45 yr	C_{ss} thalamate	500 cpm/ml
Body weight	70 kg	Infusate thalamate concentration	500000 cpm/ml
Serum creatinine	120 µmol/L		
Sex	female		

$$CL_{cr}\,(ml/min) = \frac{(140-45) \times 70\ (kg)}{120\ (\mu mol/L) \times 0.815} \times 0.85 = 58\ ml/min$$

$$R_{thalamate}\,(ml/min) = \frac{500\ (cpm/ml) \times 58\ (ml/min)}{500000\ (cpm/ml)} = 0.058\ ml/min = 3.48\ ml/hr$$

$$D\,(ml) = \frac{C_{ss}\,(cpm/ml) \times V_d\,(ml)}{Infusate\ concentration\ (cpm/ml)} = \frac{500 \times 17500}{500000} = 17.5\ ml$$

Clearance protocol

Following a 90-minute equilibration phase, urine was collected during two 45 minute periods. These collection periods were bracketed by blood samples. The

average of the two collection periods was used for further analysis. The clearances of thalamate and hippuran based on urine collection were then calculated from:

$$CL_{urine} = U_x V / P_x$$

where U_x represents the urinary concentration of solute x, P_x the plasma concentration of solute x and V the volume of the urine output. The endogenous creatinine clearance was measured simultaneously.

The clearances of ^{125}I-thalamate and ^{131}I-hippuran was also calculated by dividing the infusion rate of the clearance markers by the plasma concentration of these markers at steady state (CL_{plasma}). The clearance of (CL_{plasma}) was calculated using:

$$CL_{plasma} = R \times St_x / (P_x - P_0)$$

in which R is the infusion rate (ml/min), St_x the standard (counts of the infusion solution; cpm/ml) while P_x and P_0 represent the counts of the plasma sample at time x and a plasma sample taken just before the start of the infusion (cpm/ml) respectively. All measures of GFR and ERPF were corrected for a body surface area of 1.73 m^2.

Statistics

The data were analyzed for all patients combined and for each diagnostic group separately. We also stratified the patients according to renal function (GFR >100, 61-100, 30-60 and <30 ml/min/1.73 m^2) and repeated the analysis. The achievement of steady state was evaluated using a repeated measures ANOVA, comparing plasma levels of iothalamate at 90, 135 and 180 minutes after the start of the infusion. If no time effect was observed steady state was considered to have been obtained. When multiple groups were compared a oneway ANOVA was used. If a significant F-value was observed, the Student-Neuman-Keuls test was used for further multiple comparisons. The agreement between the various methods for the determination of GFR was assessed using a graphical method described by Bland and Altman [8], in which a plot of differences against their means is made. All data are means with SEM indicated in parentheses.

Results

Achievement of steady state

The plasma levels of ^{125}I-iothalamate and ^{131}I-orthoiodohippurate are shown in Table 3. Mean plasma thalamate and hippurate levels did not significantly change after the 90-minute equilibration period (repeated measures ANOVA) and thus a steady state situation was achieved in all patient groups. Despite adjustments in the infusion rates, steady state concentrations increased as GFR decreased. The achievement of steady state was not influenced by depressed renal function as a steady state was also achieved in patients with severely reduced renal function (GFR $<$ 30 ml/min/1.73 m^2).

Comparison of estimates and measurements of GFR

Renal function varied markedly between the various groups of patients (Table 4). GFR based on measurements of urine collection and plasma concentration ranged from 40 (2) in heart transplant recipients to 131 (4) ml/min/1.73 m^2 in patients with diabetes. When comparing the means in all 212 patients, no significant differences were observed between the results obtained with urine collection and the results obtained by dividing infusion rate by plasma level (78 (3) and 79 (3) ml/min/1.73 m^2 respectively). Mean endogenous creatinine clearance (CLCr) was significantly higher than CL_{urine}Thal (91 (3) ml/min/1.73 m^2; $p < 0.05$) and GFR as estimated by means of Cockcrofts equation was significantly lower than CL_{urine}Thal (72 (2) ml/min/1.73 m^2; $p < 0.05$).

When comparing these clearances within the individual diagnostic groups, no differences were found between CL_{urine}Thal and CL_{plasma}Thal. CLCr however, was significantly higher than CL_{urine}Thal in HTX and KTX patients. These differences where also reflected by, non-significant, trends towards a higher CLCr compared to CL_{urine}Thal in patients with a GFR below 60 ml/min/1.73 m^2 (Table 4). Interestingly, mean GFR as estimated by Cockcrofts equation was significantly lower than CL_{urine}Thal in the IDDM group only (96 (2) vs. 131 (4) ml/min/1.73 m^2; $p < 0.05$). This was also the case in the patient group with GFR $>$ 60 ml/min/1.73 m^2, most likely as a result of the high number of IDDM patients in this group.

Table 3 Plasma levels of ^{125}I-thalamate and of ^{131}I-hippurate

		Thalamate							Hippurate cpm/ml						
	n =	90	min	135	min	180	min	F value	90	min	135	min	180	min	F value
Diagnosis															
Heart transplantation	69	1146	(37)	1055	(34)	1040	(34)	n.s.	317	(15)	333	(15)	331	(17)	n.s.
Kidney transplantation	51	1152	(69)	1131	(60)	1160	(68)	n.s.	375	(27)	391	(28)	399	(30)	n.s.
Diabetes mellitus	74	721	(23)	689	(22)	684	(22)	n.s.	194	(7)	210	(7)	197	(7)	n.s.
Nephrotic syndrome	18	1107	(62)	1072	(63)	1069	(63)	n.s.	238	(21)	238	(21)	256	(24)	n.s.
$CL_{urine}Thal$ *(ml/min/1.73 m^2)*															
>100	70	696	(21)	664	(20)	658	(20)	n.s.	195	(8)	210	(8)	196	(8)	n.s.
61–100	37	982	(41)	964	(42)	979	(43)	n.s.	275	(14)	287	(15)	288	(15)	n.s.
30–60	75	1130	(32)	1051	(30)	1045	(32)	n.s.	316	(14)	327	(15)	329	(17)	n.s.
<30	30	1429	(119)	1380	(111)	1388	(114)	n.s.	434	(38)	465	(38)	476	(37)	n.s.
Total	212	993	(27)	944	(26)	944	(27)	n.s.	289	(19)	304	(18)	302	(19)	n.s.

Values are mean (SEM); F-values were calculated using an ANOVA for repeated measurements.

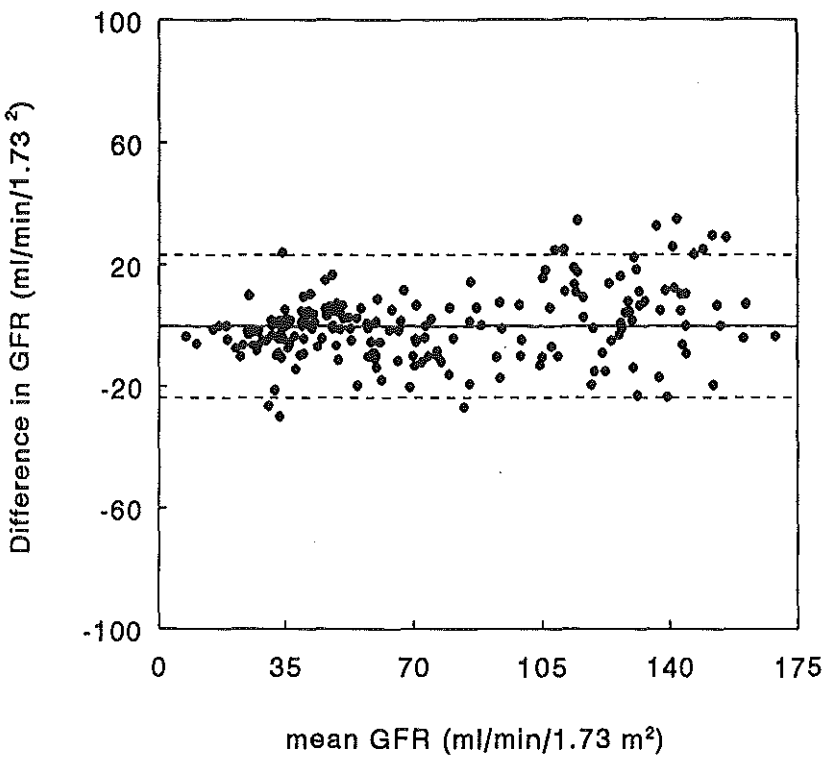
Table 4 Estimates of GFR (ml/min/1.73 m^2)

	n =	CL_{urine} Thal	CL_{plasma} Thal	CLCr	Cockcroft	F Value
Diagnosis						
Heart transplantation	69	40 (2)	41 (1)	53 (2)*	43 (1)	<0.05
Kidney transplantation	51	61 (3)	67 (4)	85 (4)*	76 (4)	<0.05
Diabetes mellitus	74	131 (4)	124 (4)	134 (4)	96 (2)*	<0.05
Nephrotic syndrome	18	67 (12)	70 (11)	68 (15)	72 (11)	n.s.
CL_{urine} Thal (ml/min/1.73 m^2)						
>100	70	138 (3)	130 (3)	138 (3)	101 (2)*	<0.05
41–60	37	77 (2)	82 (2)	100 (3)	87 (4)	n.s.
30–60	75	45 (1)	46 (1)	63 (2)	52 (2)	n.s.
GFR <30	30	21 (1)	28 (2)	30 (2)	30 (2)	n.s.
Total	212	78 (3)	79 (3)	91 (3)*	69 (2)*	<0.05

Values are mean (SEM); CL_{urine} Thal = urinary clearance of Thalamate, CL_{plasma} Thal = plasma clearance of thalamate, CLCr = creatinine clearance, Cockcroft = estimation of GFR by Cockcrofts equation; F-values were calculated using ANOVA and * = significance at $p < 0.05$ using the Student-Neuman-Keuls test.

Filtration fraction as measured by both urine and plasma clearances of iothalamate and orthoiodohippurate were the same in all groups of patients studied (Table 5).

Fig.1

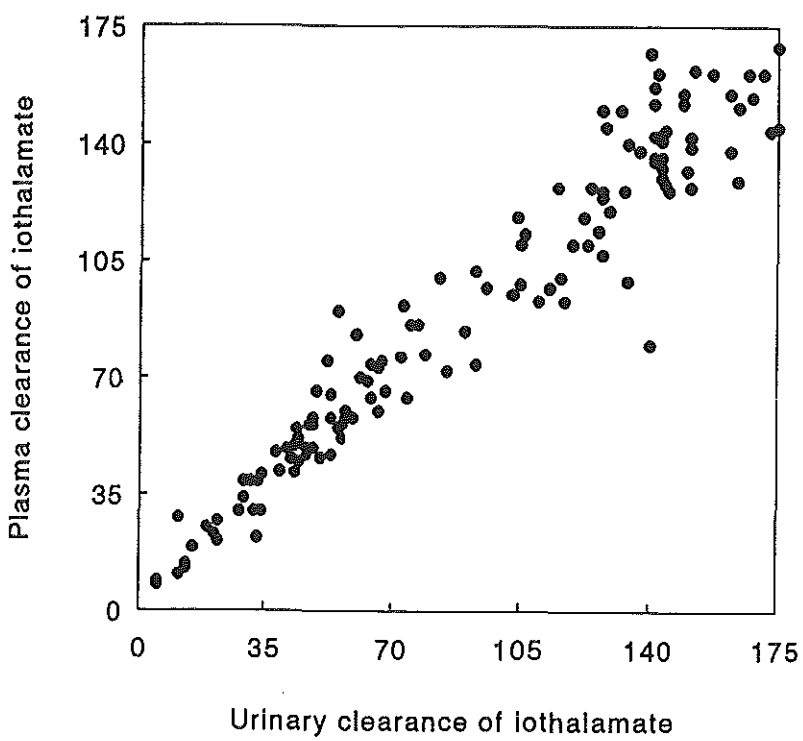


Relationship between the difference (CL_{urine}Thal-CL_{plasma}Thal) and the mean ([CL_{urine}Thal+CL_{plasma}Thal]/2) of the urinary and plasma clearances of iothalamate.

Discussion

The results of this study indicate that steady state concentrations of iothalamate and orthoiodohippurate can be achieved using a simple pharmacokinetic model based on Cockcrofts equation. Even when renal function was severely depressed, a steady state was obtained within 90-135 minutes. In our study, CL_{urine}Thal and CL_{plasma}Thal showed a good agreement as the mean difference between both methods averaged -0.23 ml/min/1.73 m^2 (95% confidence interval -1.8 to 1.4 ml/min/1.73 m^2). The relationship between the difference and the mean of

Fig. 2

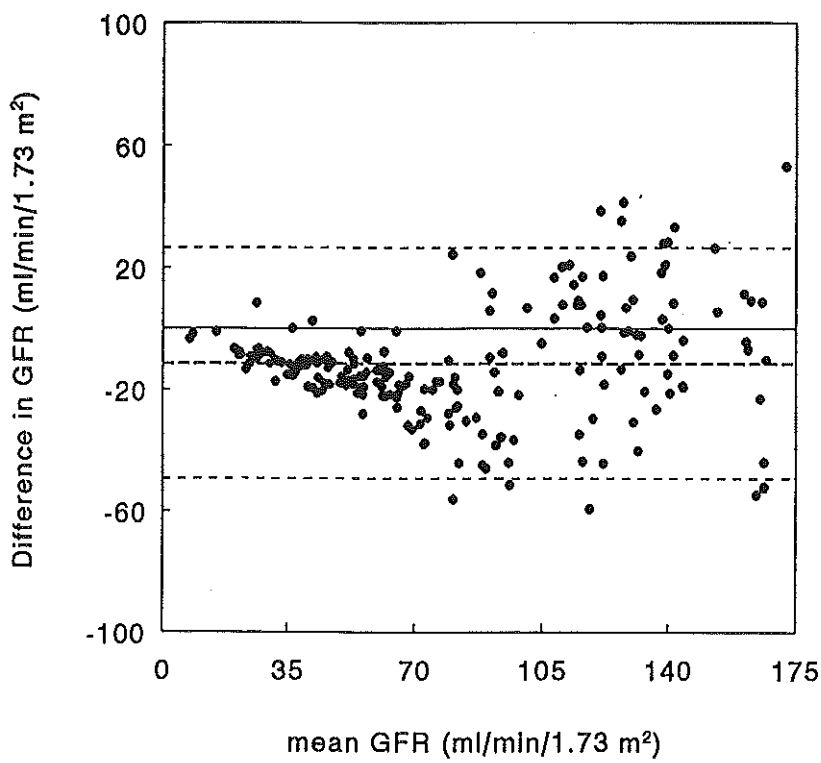


Relationship between CL_{urine}Thal and CL_{plasma}Thal.

CL_{urine}Thal and CL_{plasma}Thal is shown in Fig. 1. The relation between CL_{urine}Thal and CL_{plasma}Thal is shown in Fig. 2. The lack of differences in filtration fraction derived from both methods (Table 5) also supports the association between urine and plasma clearance methods as both are independent of the collection of urine samples.

On the whole, CLCr was higher than CL_{urine}Thal (11.5 ml/min/1.73 m^2 (95% confidence interval 8.6 to 14.4 ml/min/1.73 m^2; Fig. 3). As could be expected, CLCr overestimated GFR by 12 % in patients with severely depressed renal function. This is a well know phenomenon which is due to increased tubular

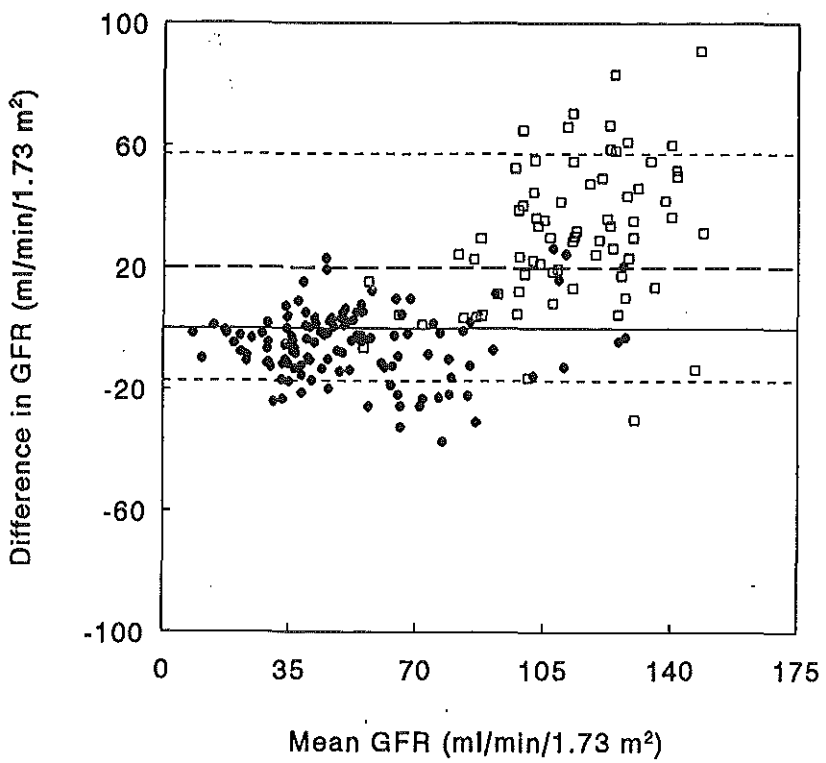
Fig. 3



Relationship between the difference and the mean of the urinary clearances of iothalamate and creatinine.

excretion of creatinine with declining renal function [9]. At higher levels of GFR, although mean CLCr was equal to CL_{urine}Thal, the variance of the difference was higher. The estimation of GFR according to the method of Cockcroft showed a good agreement with CL_{urine}Thal in non-diabetic patients only (Fig 4). In diabetics, CL_{urine}Thal was approximately 35% higher than was expected from the calculations. A possible explanation for this discrepancy could be hyperfiltration which is known to exist in 25% of diabetic patients [10]. Therefore, the formula of Cockcroft does not seem to be reliable in patients with diabetes. The error in estimation of GFR and therefore thalamate dosage, did not affect the ability to

Fig. 4



Relationship between the difference and the mean of the urinary clearance of iothalamate and the estimation of GFR by means of Cockcroft's equation. Patients with diabetes mellitus are indicated as open squares, whereas all other patients are depicted by closed circles.

achieve a steady state as renal function was not depressed in these patients and the plasma half-life of thalamate was therefore relatively short.
With slight modifications, this method is suited equally well for the determination of dosages of non-labelled clearance markers such as inulin.
We conclude that, using a simple pharmacokinetic model based on Cockcroft's equation, a steady state plasma concentration of iothalamate can be achieved, even in patients with severely depressed renal function. In patients with diabetes mellitus, the estimation of GFR by means of Cockcroft's equation underestimates true GFR and should therefore be avoided.

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Chapter 3

Contrasting response to cyclosporin in refractory nephrotic syndrome

R Zietse, GJ Wenting, P Kramer, P Mulder, MADH Schalekamp and W Weimar.
Clinical Nephrology 1989;31:22-25.

Abstract

We studied the effects of cyclosporin A (CsA), given for three months, in 14 patients with nephrotic syndrome refractory to treatment with prednisone and/or other immunosuppressants. CsA was given in a starting dose of 6 mg/kg and plasma trough levels (RIA) were kept between 50 and 150 ng/ml. Diagnoses included: idiopathic membranous glomerulonephritis (n = 6), focal segmental glomerulosclerosis (n = 3), minimal change disease (n = 3) and membrano-proliferative glomerulonephritis (n = 2). Three patients with non-immunologically mediated nephrotic syndrome due to Alport's syndrome were studied as well. Considering all patients and diagnostic groups together, proteinuria decreased from 9.0±4.3 to 4.7±3.8 g/24 h during CsA treatment (mean ± SD; $p<0.01$). However, serum creatinine increased from 121.8 ± 60.5 to 150.4±64.6 mol/l ($p<0.01$) and glomerular filtration rate as estimated by 24-hour creatinine clearance fell from 85.5±33.7 to 72.1±37.2 ml/min ($P<0.05$). When compared to other diagnostic groups, fractional excretion of protein, i.e. protein excretion corrected for changes in glomerular filtration rate, fell in MCD and IMGN only (ANOVA, $p<0.05$). We conclude that CsA reduced proteinuria in patients with refractory nephrotic syndrome. In the majority of these patients this reduction could be due to a renal hemodynamic, rather than an immuno-

modulatory effect of the drug. Only in MCD and IMGN the latter action of the drug may be of importance.

Introduction

In the management of proteinuria in the idiopathic nephrotic syndrome steroids and immunosuppressants have been widely advocated [1]. The improvement during immunosuppression suggests that the increased glomerular permeability to proteins may be caused by an immunological derangement. However not all patients respond. Persistent nephrotic syndrome is a debilitating condition, as infections may supervene and progression of the disease can lead to terminal renal insufficiency. Moreover, prolonged unsuccessful treatment with high doses of steroids is associated with severe adverse effects. Cyclosporin is a new and potent immunosuppressant. We are now witness to a proliferation of the use of

Table 1

Clinical patient data, proteinuria, creatinine clearance and serum albumin before and after 3 months of CsA in 17 patients with refractory nephrotic syndrome.

Patients	Diagnosis	Sex	Age	Baseline Protein excretion	C_{Cr}	Serum albumin	After 3 months of CsA Protein excretion	C_{Cr}	Serum albumin
		m/f	yr	g/24 h	ml/min	g/l	g/24 h	ml/min	g/l
1	IMGN	f	60	3.6	32.3	23	1.2	25.3	24
2	IMGN	m	62	8.5	31.9	30	4.3	29.8	31
3	IMGN	m	30	12.6	123.4	34	4.0	133.0	38
4	IMGN	m	26	13.3	117.7	29	6.8	91.0	36
5	IMGN	m	38	16.0	132.5	20	3.8	127.9	29
6	IMGN	m	20	16.9	51.2	22	7.9	46.5	31
7	FSGS	f	24	6.0	101.3	26	5.5	53.6	28
8	FSGS	m	35	8.7	62.4	24	7.8	37.9	26
9	FSGS	f	25	13.3	33.3	19	16.2	23.9	19
10	AS	m	22	3.4	82.9	35	1.2	26.8	41
11	AS	m	25	7.9	69.4	37	6.1	47.5	40
12	AS	m	23	8.4	93.7	33	5.5	65.1	43
13	MCD	m	35	4.5	77.8	21	0.2	55.8	37
14	MCD	m	20	4.6	97.3	22	0.0	108.3	44
15	MCD	m	21	5.9	132.2	32	1.1	123.0	42
16	MPGN	m	48	5.8	124.3	30	4.2	98.4	37
17	MPGN	f	35	14.1	89.8	26	5.2	109.3	32
			Mean	9.0	85.5	27.2	4.7	72.1	34.0
			SD	4.3	33.7	5.5	3.8	37.2	7.0
			p				0.0005	0.0133	0.0003

this drug for a variety of diseases characterized by immuno inflammation like uveitis, psoriasis, rheumatoid arthritis and diabetes. Several lines of evidence have indicated that cyclosporin also has an effect on proteinuria in patients refractory to conventional immunosuppression [2,3], but for the moment it is unclear whether the effects of the drug are due to immune modulation or to some other action, such as renal vasoconstriction and depression of GFR. To differentiate between the two possible modes of action of CsA we studied the effects of CsA on fractional protein excretion in 14 patients with a refractory nephrotic syndrome. We included 3 patients with Alport's syndrome (AS) since changes in protein excretion in this syndrome are not likely to result from the immunosuppressive actions of CsA.

Material and methods

Patients

We studied 17 patients aged 20 to 62 years, median 25 years (13 male, 4 female). Of these patients 14 had an idiopathic nephrotic syndrome refractory to prednisone and/or other immunosuppressants. Histological diagnoses were: idiopathic membranous glomerulonephritis (IMGN), focal segmental glomerulosclerosis (FSGS), minimal change disease (MCD), and membrano-proliferative glomerulonephritis (MPGN) (Table 1). We also included 3 patients with Alport's syndrome. In the 17 patients studied, protein excretion ranged from 3.5 to 16.9 g/24 h/1.73 m^2. Serum creatinine levels ranged from 64 to 311 μmol/l; the median was 106 μmol/l.

Methods

After a one-month "washout" period in which no immunosuppressants were prescribed, patients received CsA 6 mg/kg, given in two divided doses for three months. Plasma trough levels as measured by radioimmunoassay (CYCLO-Trac, Incstar, Stillwater, USA) were kept between 50 and 150 ng/ml. Patients were followed for one month after discontinuing treatment. Two 24-h urine collections

were obtained each month. Serum samples were collected at the same visit for the determination of creatinine, total protein, albumin and cholesterol levels. The average of the two urine collections was used for calculation of endogenous creatinine clearance and fractional excretion of protein.

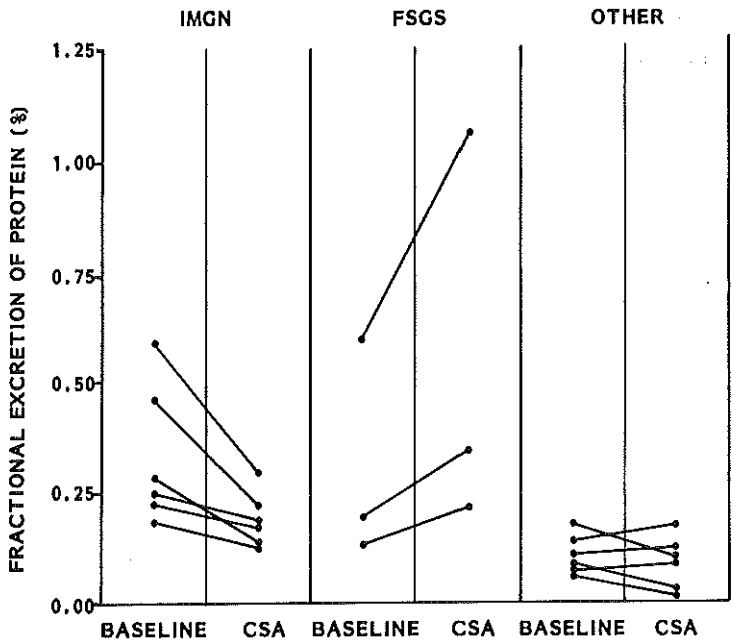
Statistical analyses

Baseline values and values after three months of CsA were compared by means of Student's t-test for paired observations. The changes in protein excretion, fractional excretion of protein and C_{Cr} in the various diagnostic groups were compared by analysis of variance. To correct for a regression effect the baseline level was entered as a covariate. When a significant difference was observed, the Tukey procedure was used for multiple range testing [4]. Data are expressed as mean$\pm$SD.

Results

CsA was well tolerated, and all patients completed the protocol. Two patients experienced minor gastrointestinal complaints, while blood pressure rose slightly in three other patients. After three months of treatment with CsA, proteinuria decreased from 9.0$\pm$4.3 to 4.7$\pm$3.8 g/24 h ($p=0.0005$, Table 1). Serum protein and albumin levels rose from 49.9$\pm$5.6 to 57.8$\pm$7.4 g/l ($p=0.0005$) and from 27.2$\pm$5.5 to 34.0$\pm$7.0 g/l ($p=0.0003$), respectively. Serum creatinine levels rose from 121.8$\pm$60.5 to 150.4$\pm$64.6 mol/l ($p=0.006$). Serum creatinine fell from 10.8$\pm$4.1 to 7.7$\pm$3.0 mmol/l ($p=0.014$) after three months of CsA. C_{Cr} fell from 85.5$\pm$33.7 to 71.2$\pm$37.2 ml/min ($p=0.013$) and fractional excretion of protein did not change during treatment with CsA. When the separate diagnostic groups were compared, the decrease in proteinuria was greater in patients with IMGN ($p<0.05$) and less in those with FSGS ($p<0.05$). When compared to other groups fractional excretion of protein decreased in patients with MCD and IMGN ($p<0.05$) and increased in patients with FSGS ($p<0.05$) (Figure 1). One month after discontinuing treatment all parameters returned to baseline levels,

Fig. 1



Fractional clearance of protein in 15 patients with nephrotic syndrome due to membranous glomerulonephritis (IMGN), focal segmental glomerulosclerosis (FSGS) and other diseases (Alport's syndrome and minimal change disease), before and after three months of CsA.

except in one patient with MCD in whom the reduction in proteinuria lasted for two months after discontinuing CsA.

Discussion

The results of our study demonstrate that in patients with a nephrotic syndrome unresponsive to conventional immunosuppressants proteinuria can be decreased by treatment with CsA. The change in proteinuria resulted in an increase of serum protein and albumin and a decrease in serum cholesterol levels, thus

indicating that CsA had a favourable effect on the protein status. As renal function was impaired during treatment with CsA, changes in renal hemodynamics may well have contributed to the observed decrease in proteinuria. This is especially likely to be the case in AS as it seems unlikely that immunosuppression can reverse the structural changes in the basement membrane in these patients. A similar mechanism may partly explain the antiproteinuric effect of indomethacin [5]. Although in the group as a whole fractional excretion of protein remained unchanged, when compared to other diagnostic groups it decreased in MCD and IMGN, and increased in FSGS. This indicates that both in MCD and IMGN the decrease in proteinuria is not in proportion to the decrease in GFR. Therefore an action of CsA other than its effect on renal blood flow may be responsible for its effect in these diseases. There is mounting evidence that IMGN is a largely immune mediated disease [6]. CsA modulates the immunoregulatory response of T cells by blocking the synthesis and release of lymphokines [7]. These actions of CsA may be responsible for the observed effects in patients with IMGN. Furthermore there is some evidence that the negative charge content of the basement membrane, which is a major determinant of protein excretion, may be altered by lymphokines [8]. IMGN is a disorder which is frequently characterized by a precarious course of progression, varying from spontaneous remissions to end-stage renal disease [9]. CsA may especially be of use in the group of patients with a less benign course.

At present it remains unclear how long treatment with CsA should be continued. In the present study treatment was stopped after three months. As protein excretion returned to baseline levels within one month of discontinuing treatment the effect of CsA appeared to be temporary. Recent data however suggest that continued treatment with CsA may further decrease protein excretion in patients with IMGN [10]. We therefore advocate a controlled trial of prolonged treatment with CsA in patient with IMGN. We conclude that CsA reduced proteinuria in patients with refractory nephrotic syndrome. In the majority of these patients this reduction could be due to a renal hemodynamic, rather than an immuno-modulatory effect of the drug. Only in MCD and IMGN the latter action of the drug may be of importance.

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Chapter 4

Effects of cyclosporine on glomerular barrier function in nephrotic syndrome

R Zietse, GJ Wenting, P Kramer, MADH Schalekamp and W Weimar. Clinical Science 1992;82:641-650

Abstract

To elucidate the mechanisms by which cyclosporine A (CsA) diminishes proteinuria, we studied 20 patients with severe nephrotic syndrome. Biopsy established pathologies included minimal change disease (MCD, n=5), membranous glomerulopathy (MG, n=6), membranoproliferative glomerulonephritis (MPGN, n=5) and focal segmental glomerulosclerosis (FSGS, n=4). Before, at the end of a 90-day course of CsA, and finally one month after stopping CsA we determined 24-h protein excretion. Measurements of GFR, ERPF, fractional clearance rates of albumin and immunoglobulins with different charge and the transglomerular sieving of uncharged dextrans of broad size distribution were used to study the effects of CsA on renal perfusion and the glomerular filtration barrier. The findings were analyzed with a theoretical model of solute transport.

Among the different forms of glomerulopathy the response to low dose CsA (trough level 35 ng/ml) varied markedly. In MCD, proteinuria decreased from 9.5 ± 3.1 to 1.3 ± 0.2 g/24h (mean$\pm$SEM, $p < 0.01$). This response was due to restoration of charge selectivity of the glomerular barrier. The depressed value of the glomerular permeability coefficient also returned to normal. GFR, ERPF and renal vascular resistance did not change. Proteinuria returned after stopping

CsA although it did not reach pre-treatment levels. In MG, proteinuria fell from 9.9 ± 1.5 to 1.8 ± 0.3 g/24h ($p < 0.01$). Changes in protein excretion and dextran sieving were compatible with an increase in glomerular permselectivity and a decrease in filtrate flow through the "shunt" pathway. GFR was maintained although ERPF fell significantly. Proteinuria relapsed after stopping CsA. In MPGN and FSGS proteinuria did not respond to CsA although CsA exerted important hemodynamic effects.
In MCD and MG cyclosporine exerts its beneficial effects on proteinuria through changes in the properties of the glomerular barrier, resulting in increased charge and size selectivity respectively.

Introduction

In recent years a role has emerged for cyclosporine-A in the treatment of nephrotic syndrome. Favourable effects of this drug have been reported in minimal change disease [1], the asian form of IgA nephritis [2] and membranous glomerulopathy [3]. As yet, the precise mode of action of cyclosporine in these disorders is the subject of vivid debate. Cyclosporine is a potent suppressor of T-cell derived lymphokines [4] which could play a role in the permeability of the glomerular basement membrane under pathological conditions [5]. On the other hand, CsA is known to exert distinct effects on renal perfusion and filtration through constriction of the afferent glomerular arteriole [4] which could, at least in part, contribute to cyclosporine's antiproteinuric action by reducing net ultrafiltration pressure.
In an attempt to delineate the effects of cyclosporine on ultrafiltration in more detail, we studied renal hemodynamics and glomerular basement membrane permeability in 20 patients with various forms of nephrotic syndrome prior to and after a 12-week course of cyclosporine therapy. The clearances of thalamate, hippurate, neutral dextrans of graded size, and the urinary excretion of albumin, IgG and IgG_4 were used to study the effects of cyclosporine on renal perfusion and the glomerular filtration barrier.

Methods

Patients and study design

Adult patients with nephrotic syndrome and specific glomerular pathology that had been diagnosed by renal biopsy and who had no evidence of underlying systemic disease were eligible for the ongoing open trial, in which the antiproteinuric effects of cyclosporine A (CsA) are investigated. The criteria for inclusion were a rate of urinary protein excretion of ≥ 3.5 g per day and a creatinine clearance of ≥ 40 ml/min. The use of immunosuppressive agents, angiotensin converting-enzyme inhibitors or non-steroidal anti-inflammatory drugs was not allowed in the two months before entry or during the three months of the study.

The present report includes 20 patients. A diagnosis of minimal change disease (MCD) was made in five patients (one patient was steroid resistant and four were frequent relapsers), membranous glomerulopathy (MG) in six, membranoproliferative glomerulonephritis (MPGN) in five and focal segmental glomerulosclerosis (FSGS) in four. The clinical patient characteristics are shown in Table 1. All patients had evident peripheral oedema.

Table 1

Clinical characteristics of the patients

Patient no.	Diagnosis	Sex (M/F)	Age (years)	Proteinuria (g/24 h)	Serum creatinine concn. (μmol/l)	Serum albumin concn. (g/l)
1	MCD	M	33	14.7	78	17
2	MCD	M	23	8.7	69	15
3	MCD	F	29	7.4	79	15
4	MCD	F	22	5.9	50	26
5	MCD	F	50	14.7	77	18
6	MG	M	40	8.6	95	23
7	MG	F	71	9.6	104	29
8	MG	M	62	5.2	74	25
9	MG	M	30	12.9	87	20
10	MG	M	34	6.7	83	34
11	MG	M	45	16.3	133	21
12	MPGN	M	39	11.7	88	16
13	MPGN	M	74	18.6	219	18
14	MPGN	M	62	14.4	139	27
15	MPGN	F	47	11.6	254	23
16	MPGN	M	23	10.7	77	26
17	FSGS	F	19	5.9	83	18
18	FSGS	F	43	12.3	83	25
19	FSGS	M	28	10.0	76	21
20	FSGS	M	41	12.0	87	24

The study period consisted of a two month observation period followed by a three month CsA treatment period. Cyclosporine was taken at meal-times in two separate doses. The starting dose was 6 mg/kg per day. Subsequent maintenance CsA therapy was adjusted to achieve trough levels of immunoassayable CsA in plasma around 50 ng/ml. A specific monoclonal antibody (Cyclotrac, Incstar, Stillwater, USA) was used to characterize the parent drug. Patients were monitored at two-week intervals for one month and every four weeks until the third month or more frequently if indicated by their clinical status. Routine biochemistry, CsA trough levels and twenty-four hour urinary protein excretion were assessed at these visits. Eight healthy volunteers who were normotensive, free of known renal disease, and devoid of clinically measurable proteinuria served as a control group for the differential dextran clearances.

Procedures

Each patient was studied twice, at the start of the trial and following three months of treatment with CsA, after giving informed consent to the study procedures that had been approved previously by the Ethical Committee of the Dijkzigt Hospital. All patients were studied using the following clearance protocol.

After a light breakfast patients drank tap water, 20 ml/kg body weight, in 20 minutes. Plastic cannulas were then inserted into an antecubital vein of each arm. One arm was used for the infusion of dextrans and radiolabeled clearance markers, while blood samples were drawn from the other arm. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were determined by a constant infusion technique, by measuring the renal clearances of ^{125}I-iothalamate and ^{131}I-orthoiodohippurate (Amersham, UK) respectively. The priming dose and sustaining infusion rate were adjusted for renal function using the following method. The GFR and therefore the clearance of iothalamate was estimated using the equation of Cockcroft and Gault [6]. The clearance of iodohippurate was estimated to be fivefold higher, assuming a filtration fraction of approximately 0.20. Infusion rates was then calculated to yield desirable steady state levels. A suitable priming dose was calculated from the estimated steady state levels and the volume of distribution. During the study period all patients remained in supine position but were allowed to stand in order to pass urine.

After a one hour equilibration period the bladder was emptied by voiding. Following a slow injection of 200 mg of dextran-10 (Promiten®, NPBI, Emmercompascuum, The Netherlands) in order to avoid anaphylactic reactions, 70 mg/kg dextran-70 (Macrodex®) and 70 mg/kg dextran-40 (Rheomacrodex®) were administered in a 10 minute infusion. Urine was collected during three carefully timed 30 minute intervals. Plasma and urine counts of ^{125}I-iothalamate and ^{131}I-iodohippurate were measured in a gamma scintillation counter. The standard formula was used to calculate the clearance values. The mean GFR and ERPF were calculated from the three clearance periods. Renal vascular resistance (RVR) was calculated as
[(MAP-10) x (1-Ht)/ERPF] x 22.16 (kdyn.s.cm^{-5}). During the last collection period three plasma samples were drawn at the beginning, middle and end respectively. These samples were combined in order to obtain an "average" plasma concentration of dextran. Fractional clearances (F_{CL}), or in the case of dextran sieving coefficients, of macromolecules (M) were calculated using the equation:

$$F_{CL}M = [(U/P)M / (U/P)Thalamate]$$

in which U and P denote the urinary and plasma concentrations of the macromolecule and thalamate. In the urine and plasma samples used for the measurement of dextrans we also measured albumin, IgG and IgG_4 concentrations. The selectivity index (SI) was calculated as CL IgG/CL albumin. In general, proteinuria is considered to be selective when SI is below 0.2. As IgG and IgG_4 are differently charged at physiological pH, the CL IgG/CL IgG_4 can be used as an indicator of charge selectivity (charge index; CI) [7].

Laboratory methods

Blood pressure was determined with an oscillometric device (Accutorr, Datascope Corp, Paramus, New Jersey, USA). The means of 5 consecutive readings following a 15 minute "run-in" period were used for analysis.
Serum creatinine concentrations were measured using a modified Jaffé method. Total urinary protein concentrations and albumin concentrations in plasma and urine were measured using an immunoturbidimetric assay. For the determination of IgG and IgG_4 in plasma and urine a sandwich radioimmunoassay was

developed. In brief, Maxisorb test tubes (Nunc, Roshilde, Denmark) were coated with a monoclonal anti-IgG or anti-IgG_4 (MH16-01M and MH164-1 respectively; Central Laboratory for Bloodtransfusion (CLB), Amsterdam, The Netherlands). After overnight incubation with sufficiently diluted samples the tubes were washed carefully. Another monoclonal anti-IgG or IgG_4 (MH16-02M or MH164-4, CLB) radiolabeled with ^{125}I was added and after washing, the tubes were assayed for IgG or IgG_4 using reference serum (0-001, CLB) as the standard. The sensitivity of both assays was 1 ng/ml. Interassay coefficients of variation were 6.4 and 7.5% respectively. Dextran was assayed after protein-free filtrates of plasma and urine had been separated into narrow fractions by gel-permeation chromatography using the method described by Granath et al. [8]. A sephacryl S-300 column (Pharmacia, Upsala, Sweden) was used of 180 ml bed volume and 90 cm in length. The eluent was a 0.01 M Tris buffer with 0.15 M NaCl and 1 Mm EDTA at pH 7.0. Blue dextran was used to determine the void volume (V_o) and the column was calibrated using dextran T10, T40 and T70. The fractional volume available to the solute (K_{AV}) was then calculated from :

$$K_{AV} = (V_e - V_o)/(V_t - V_o)$$

where V_e is the elution volume of the solute and V_t is the total bed volume of the gel column. Effective molecular radii for the individual dextran fractions were calculated from K_{AV}. After gel permeation chromatography, eluted fractions were assayed for dextran using the anthrone method of Scott and Melvin [9]. Afferent colloid osmotic pressure (π_a) was calculated using the formula of Landis and Pappenheimer [10].

Analysis of glomerular membrane pore structure

To analyze the size selective properties of the glomerular barrier we used a heteroporous model of the glomerular capillary wall as described by Deen [11]. This model has been shown to provide the most satisfactory representation of dextran sieving. In this model the major portion of the capillary wall is perforated by restrictive cylindrical pores of identical radius (r_o). In addition this model assumes a parallel "shunt pathway" that does not discriminate on the basis of dextran size and through which a small fraction of the filtrate volume (ω) passes. This fraction is not merely dependent on changes in the properties of the capillary

wall but also on intracapillary oncotic pressure. Therefore a quantity closely related to ω, but characteristic of the membrane per se, is used. This quantity (ω_0), is the fraction of the volume flux that would pass through the shunts if plasma proteins were absent. The membrane barrier to dextrans is fully characterized by r_0, ω_0 and K_f, where K_f is the product of effective hydraulic permeability and glomerular capillary surface area. An important supposition in these calculations is the value of the trans-membrane hydraulic pressure difference, ΔP. Since ΔP cannot be measured directly in humans, a value must be assumed in order to calculate the basement membrane parameters. In normal humans ΔP is predicted to be close to 35 mmHg [12], whereas in most forms of renal disease ΔP appears to be elevated to approximately 40 mmHg. We therefore calculated intrinsic basement membrane parameters using a ΔP of 35 mmHg in healthy control subjects and 40 mmHg in the various forms of glomerulopathy. In order to evaluate wether changes in the assumed value of ΔP could explain the altered dextran sieving, we varied ΔP over a 25 mmHg range and calculated the corresponding values of r_0, ω_0 and K_f.

Statistical analyses

Differences between the four diagnostic groups were analyzed using analysis of variance and, when yielding a significant F-value, followed by the Student-Newman-Keuls test. As the numbers in the separate groups were modest, in each group the difference between baseline and CsA periods was tested parametrically, using the Wilcoxon rank-sum test. Results are expressed as means$\pm$SEM.

Results

At the low doses used side-effects of CsA were minimal and consisted of a slight rise in blood pressure in some patients. No antihypertensive treatment was required. All patients completed the 12 week course of therapy according to the protocol. The mean plasma levels of CsA were comparable in all groups and averaged 34.2 ng/ml. Before treatment proteinuria was massive with no significant difference between the various diagnostic groups. The antiproteinuric

Table 2

Renal protein handling in patients with various forms of the nephrotic syndrome at baseline and after 3 months of CsA. Values are means (SEM). Statistical significance: *$P<0.05$ versus baseline; †$P<0.05$ versus MCD; ††$P<0.05$ versus MG.

	MCD		MG		MPGN		FSGS	
	Baseline	CsA	Baseline	CsA	Baseline	CsA	Baseline	CsA
Albumin excretion rate (μg/min)	4323	714*	5281	1516*	6940	9110	4858	5604
	(550)	(418)	(404)	(141)	(1036)	(1597)	(188)	(1598)
IgG excretion rate (μg/min)	64	11*	207†	51*	392†	385	130†	160
	(24)	(7)	(49)	(13)	(41)	(196)	(25)	(75)
$10^5 \times F_{CL}$ of albumin	277	22*	287	74*	893††	1606*	539†	720*
	(54)	(15)	(55)	(17)	(223)	(595)	(45)	(123)
$10^5 \times F_{CL}$ of IgG	26	3*	75†	12*	471††	495	72†	176*
	(4.4)	(1.8)	(13)	(3)	(254)	(170)	(15)	(103)
$10^5 \times F_{CL}$ of IgG_4	25	1*	41†	11*	404††	599	77†	213*
	(4.5)	(0.4)	(13)	(1.6)	(230)	(260)	(34)	(137)
SI (IgG clearance/albumin clearance)	0.158	0.299*	0.290†	0.203*	0.441†	0.341	0.292†	0.294
	(0.080)	(0.093)	(0.052)	(0.039)	(0.141)	(0.027)	(0.012)	(0.027)
CI (IgG clearance/IgG_4 clearance)	1.1	3.7*	2.3	1.2	1.9	1.7	1.2	1.2
	(0.08)	(0.93)	(0.31)	(0.12)	(0.87)	(0.45)	(0.34)	(0.19)

Table 3

Filtration dynamics in patients with various forms of the nephrotic syndrome at baseline and after 3 months of CsA. Values are means (SEM). Statistical significance: *$P<0.05$ versus baseline; †$P<0.05$ versus MCD; ††$P<0.05$ versus MG.

	MCD		MG		MPGN		FSGS	
	Baseline	CsA	Baseline	CsA	Baseline	CsA	Baseline	CsA
GFR (ml min^{-1} 1.73 m^{-2})	100	112	87†	71	53††	45*	69†	50*
	(10)	(11)	(14)	(13)	(13)	(13)	(2)	(12)
ERPF (ml min^{-1} 1.73 m^{-2})	631	496	642	419*	461††	331*	499†	308*
	(54)	(27)	(106)	(53)	(113)	(89)	(100)	(87)
Filtration fraction	0.16	0.22*	0.14	0.17*	0.15	0.14	0.15	0.17
	(0.02)	(0.01)	(0.02)	(0.02)	(0.03)	(0.01)	(0.02)	(0.02)
Mean blood pressure (mmHg)	98.5	97.0	102.1	99.9	114.2††	126.7*	98.7	113.7*
	(1.4)	(1.6)	(2.8)	(2.0)	(5.5)	(5.6)	(4.7)	(7.1)
RVR (kdyn s cm^{-5})	6.6	8.3	7.5	11.6*	11.5†	18.6*	9.1†	17.2*
	(0.6)	(1.2)	(0.8)	(0.9)	(1.2)	(1.4)	(1.1)	(1.3)
Plasma CsA concn. (ng/ml)		32.0		34.3		35.8		36.9
		(2.9)		(4.9)		(5.1)		(8.6)

effect of CsA however varied markedly.

Minimal change disease

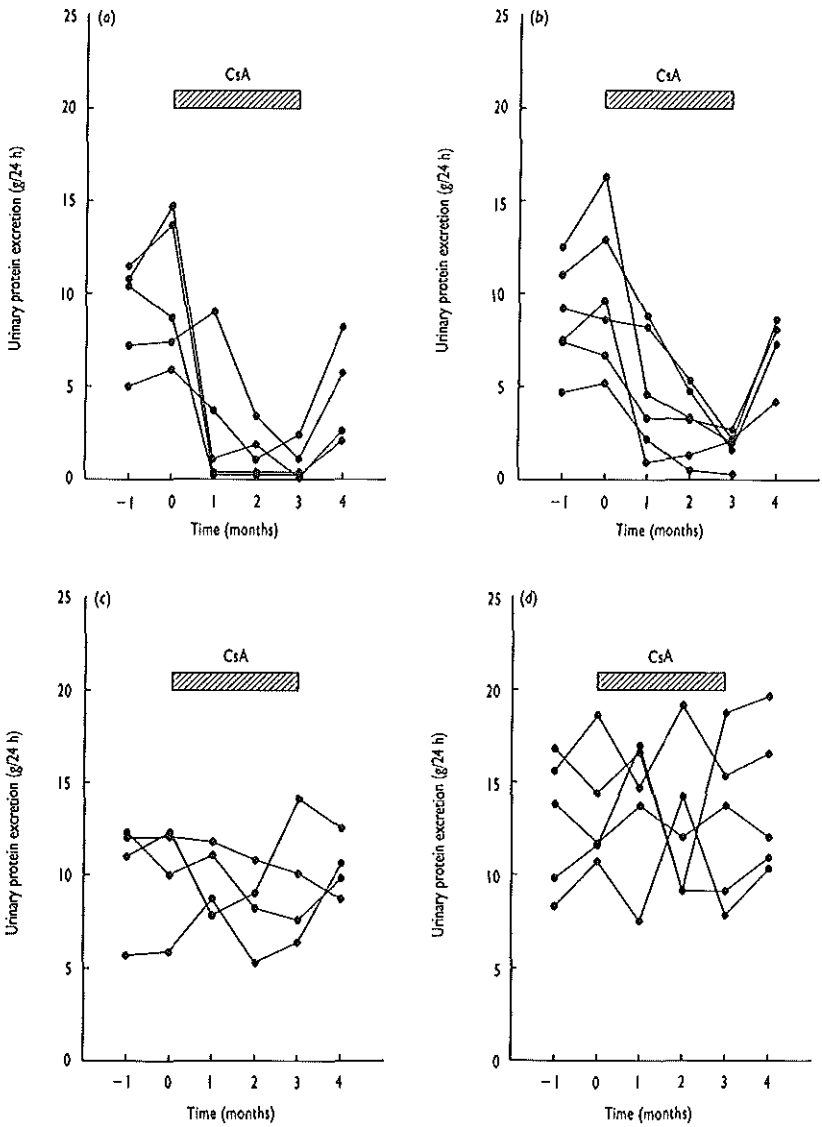
As could be expected proteinuria in these patients was highly selective. Urinary excretion rates of albumin and IgG were 4323±550 and 64±24 μg/min respectively and the index of size selectivity (SI) was 0.158±0.080 (Table 2). Renal function was not depressed as GFR and ERPF averaged 100±10 and 631 ±54 ml/min respectively (Table 3) and mean serum creatinine was 70.6±4.6 μmol/L. Blood pressure was normal in these patients. The normal size-selectivity of the proteinuria was also reflected by the baseline dextran sieving pattern as the F_{CL} of large (50-58 Å) dextrans was comparable to that of normals. However, the F_{CL} of smaller dextrans (28-32 Å) was lower than that in healthy control subjects, which is compatible with a 53% decrease in K_f (Table 4).

Following treatment with CsA, proteinuria was markedly decreased (Fig. 1) and the fractional clearances of albumin and IgG were lowered by 92 and 87% respectively and the serum albumin concentration increased from 18.2±1.8 to 36.2±3.7 g/L ($p<0.01$). Charge selectivity increased during CsA as reflected by the increase in CL IgG / CL IgG_4 (Table 2). GFR remained unaltered whereas ERPF tended to decrease although this did not reach significance (Table 3). The filtration fraction rose significantly following CsA. No changes in blood pressure or renal vascular resistance were observed. After CsA treatment the F_{CL} of dextrans of 28 and 30 Å was significantly increased in patients with MCD. No change occurred in the clearance of the larger dextrans (Fig. 2). K_f was increased from 6.3±1.9 to 9.8±1.3 $ml.min^{-1}.mmHg^{-1}$ ($p<0.05$, Table 4). When varying the assumed value of ΔP, a decrease in ΔP from 40 to 28 mmHg was necessary to explain the altered dextran sieving without changing K_f (Fig. 3). No significant changes occurred in r_0 or ω_0. Treatment with CsA was discontinued in four out of five patients after which proteinuria increased towards, although not reaching, baseline values.

Membranous glomerulopathy

The amount of proteinuria was comparable to that in MCD. However, proteinuria

Fig. 1



Proteinuria at baseline, during 3 months of treatment with CsA and 1 month after discontinuing treatment in patients with various forms of nephropathy. (*a*, MCD; *b*, MG; *c*, FSGS; *d*, MPGN) CsA was not discontinued in one patient with MCD and two patients with MG.

was nonselective as the urinary excretion rate of IgG was 207 ±49 μg/min and SI was 0.290 ±0.052 (Table 3). GFR in MG was lower than in MCD, 87 ±14 vs. 100 ±10 ($p<0.05$) and serum creatinine averaged 96 ±6.1 μmol/L. ERPF, FF and blood pressure did not differ significantly from MCD. The decrease in size-selectivity was also reflected by dextran sieving. The clearances of dextrans with a small radius where lower and those with large radii where higher when compared to normal subjects. K_f was lower and shunt flow higher than in control subjects (Table 4).

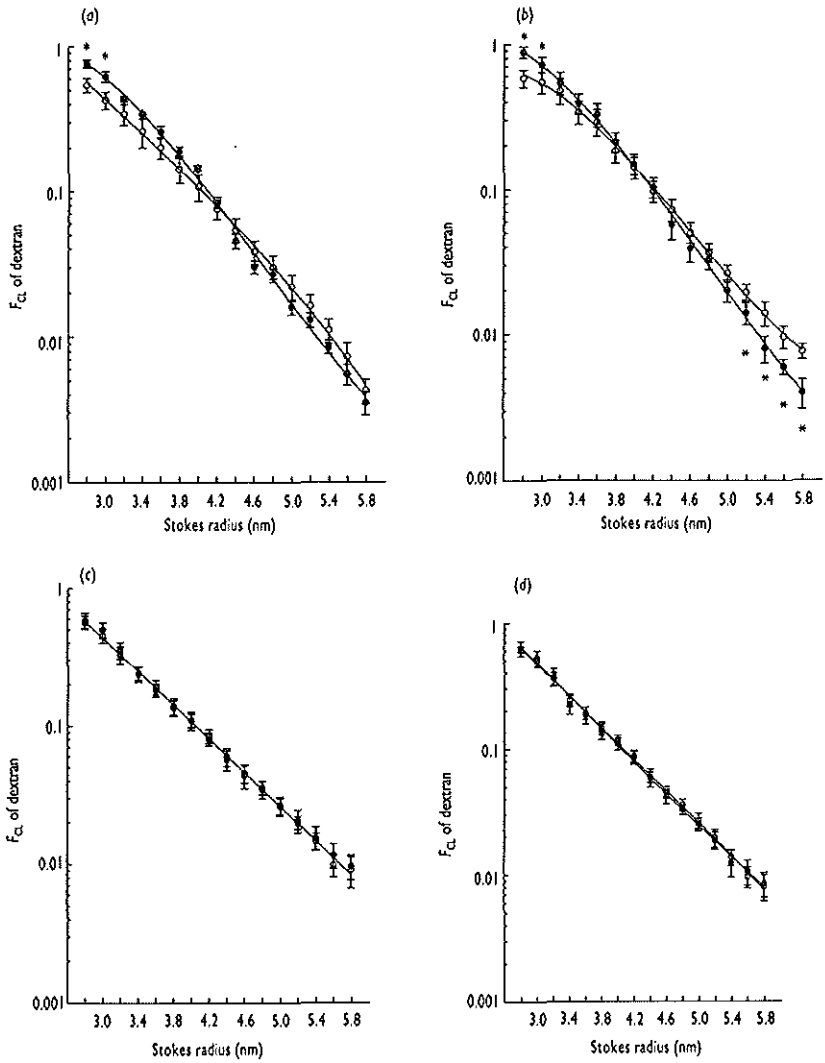
In MG CsA induced a decrease in protein excretion from 9.9±1.2 to 1.8±0.3 g/24h ($p<0.01$). The fractional clearance of albumin and IgG decreased by 68 and 84 % ($p<0.05$), respectively. Serum albumin rose from 25.3±2.0 to 37.0 ±1.2 g/L ($p<0.01$). Following CsA the selectivity index decreased by 30 % ($p<0.05$) whereas charge selectivity remained unaltered. GFR was not significantly decreased but ERPF decreased by 35% ($p<0.05$). Filtration fraction rose by 21% ($p<0.05$). Mean blood pressure remained unchanged but the renal vascular resistance was elevated by 55% ($p<0.05$). CsA significantly increased the clearance of dextrans with radii of 28 and 30 Å and decreased the clearance of those with a radius of 52 to 58 Å (Fig. 2). These findings fit in well with the observed decrease in SI. The calculated basement membrane parameters indicate that glomerular permselectivity increased as ω_0 was decreased by CsA, from 8.0 ±1.7 to 2.3±0.6 $x10^{-3}$ ($p<0.05$). As can be seen in Fig. 3, the assumption of ΔP did not markedly influence the calculated value of ω_0. K_f was not significantly altered by CsA in MG. Proteinuria increased towards baseline levels in the four patients in which treatment was discontinued (Fig. 1).

Membranoproliferative GN

In these patients proteinuria was equal to that in MCD and MG. Renal function was markedly depressed as GFR was 53±13 ml/min, a value lower than in MCD and MG ($p<0.05$). Blood pressure was higher than in these two groups ($p<0.05$). Proteinuria was non-selective (SI 0.441±0.141) and baseline dextran sieving was also compatible with decreased size selectivity.

Following treatment with CsA, GFR and ERPF were decreased by 15 and 28 % respectively and filtration fraction remained unchanged. Mean blood pressure

Fig. 2



F_{CL} of neutral dextrans measured before (○) and after 3 months treatment with CsA (●) in patients with various forms of nephropathy. (*a*, MCD; *b*, MG; *c*, FSGS, *d*, MPGN). Values are means ± SEM. Statistical significance: $^{*}P < 0.05$ versus baseline.

increased by 11% and renal vascular resistance increased by 62%. In spite of these effects on hemodynamics, no changes were observed in proteinuria and albumin or IgG excretion (Fig. 1, Table 3). The serum albumin concentrations were not altered by CsA (22.0±1.9 before and 23.4±2.6 following treatment). Also, no changes in dextran sieving or GBM permeability characteristics were observed (Fig. 2).

Focal segmental glomerulosclerosis

In the baseline situation, proteinuria was comparable to that in the other groups. GFR was lower than in MCD (70±2 ml/min, $p<0.05$) but did not differ significantly from the other patient groups. Proteinuria was non-selective as indicated by both a SI of 0.292±0.012 and a dextran sieving pattern comparable to that in MG and MPGN.

Patients with FSGS responded to CsA in a fashion similar to MPGN with significant changes in GFR, ERPF and RVR which where not accompanied by changes in protein excretion or serum albumin concentrations (Table 2, Table 3). CsA did not alter dextran sieving in FSGS.

Discussion

Since the original report by Meyrier et al. in 1986 that cyclosporine could diminish proteinuria in some nephrotic patients [13], the number of studies on this subject has steadily increased [1-3,14,15]. In particular in corticosteroid sensitive and multi-relapsing cases cyclosporine appeared to be effective [1]. However the results of many of these pilot studies are open to criticism as they differed widely in terms of objectives, population studied, cyclosporine dosage, effects on renal function and concomitant immunosuppressive treatment. Our previous findings of a pronounced effect of cyclosporine on proteinuria in patients with Alport's syndrome, a non-immunological glomerular disease, suggested that cyclosporine could operate, at least in part, without interfering with a specific immunological mechanism [16]. From experience with cyclosporine in organ

transplantation and autoimmune disorders such as uveitis [17] and psoriasis [18] it appeared that cyclosporine can induce intense, dose-dependent, renal vasoconstriction, probably at the pre-glomerular afferent arteriole. Theoretically, predominant afferent arteriolo-constriction can reduce the net ultrafiltration pressure and thereby proteinuria. Indeed, findings of Myers et al. who observed a trend towards restricted transglomerular transport of neutral dextrans of graded size in CsA-treated heart transplant recipients [19], and measurements by Barros et al. of glomerular hemodynamics in Munich-Wistar rats [20], pleaded for the case of an effect of CsA on K_f and/or trans-membrane hydraulic pressure. Thus, of the four determinants of glomerular ultraflitration
i.e. 1) rate of nephron plasma flow 2) afferent oncotic pressure 3) glomerular hydrostatic pressure gradient and 4) glomerular permeability coefficient, cyclosporine could have an effect on at least three. In the present study we deliberately opted for a relatively low-dose regimen in order to minimize renal

Table 4

Membrane parameters in control subjects and in patients with various forms of the nephrotic syndrome at baseline and after 3 months of CsA. Values are means (SEM). Statistical significance: $^{*}P < 0.05$ versus control subjects; $\dagger P < 0.05$ versus baseline. Calculations were made using a ΔP of 35 mmHg in healthy control subjects and 40 mmHg in various disease states.

	K_f (ml min^{-1} mmHg)	r_0 (nm)	$10^3 \times \omega_0$
Control subjects	13.3	5.69	1.4
	(0.9)	(0.01)	(0.1)
MCD			
Baseline	6.3*	5.57	2.5
	(1.9)	(0.01)	(0.4)
CsA	9.8†	5.53	1.4
	(1.3)	(0.01)	(0.2)
MG			
Baseline	4.8*	5.55	8.0*
	(1.6)	(0.02)	(1.7)
CsA	6.7	5.62	1.6†
	(2.0)	(0.03)	(0.6)
MPGN			
Baseline	3.5*	5.58	11.3*
	(1.4)	(0.02)	(3.2)
CsA	2.6	5.57	10.6
	(1.2)	(0.01)	(3.0)
FSGS			
Baseline	3.9*	5.49	8.9*
	(2.6)	(0.02)	(1.6)
CsA	3.0	5.62	9.2
	(2.4)	(0.03)	(2.6)

side effects. Doing so we found that the antiproteinuric effects of CsA (plasma trough levels around 35 ng/ml) varied markedly among the different forms of glomerulopathy.

In our patients with minimal change disease the antiproteinuric effects of low-dose cyclosporine were most striking as a complete remission was obtained in three out of five patients. Before cyclosporine, in the baseline untreated state, GFR, ERPF and blood pressure were normal and the massive proteinuria was highly selective. The fractional clearance of large dextrans with radii > 40 Å was comparable to that in normals. In contrast, the sieving of small dextrans was hindered. By applying our dextran sieving data to a well described and applied theoretical model of solute transport, we could explain the abnormal sieving of small dextrans in this condition by a reduction in K_f, the product of hydraulic permeability and capillary surface area. Similar findings have been reported by Bridges et al [21].

The marked reduction in protein excretion observed in MCD after cyclosporine was due to restoration of charge selectivity of the glomerular basement membrane. Serum creatinine, GFR and blood pressure did not change although ERPF and renal vascular resistance showed non-significant down- and upward trends, respectively. Filtration fraction rose significantly. Likewise, the hampered sieving of small dextrans was improved by cyclosporine although values did not reach the level observed in normal controls. Calculation of membrane parameters indicated that the raised fractional clearance of small dextrans can be accounted for by a rise of K_f. A raised K_f implies the emergence of more pores or an augmented glomerular capillary surface area. Such effects are difficult to reconcile with a vasoconstrictory effect of cyclosporine. Unfortunately, a pronounced decrease in ΔP is predicted to have similar effects on dextran sieving as a rise in K_f [22]. However, if K_f is supposed to remain unchanged after cyclosporine, a decrease in ΔP to 28 mmHg would be required to explain our results. Given the observed increase in filtration fraction, such a fall of ΔP seems very unlikely. Furthermore, it is noteworthy that comparable changes in the sieving of small dextrans in MCD were described after treatment with prednisone [23], a drug without an important hemodynamic mode of action. Current understanding of the pathophysiology of MCD focuses on a circulating "permeability" factor that somehow alters the negative charge of the glomerular

basement membrane [24]. It has been suggested that this factor could be a T-cell derived lymphokine [5,25]. As cyclosporine inhibits the production of lymphokines by T-cells [4], our findings of a recovery of charge selectivity during cyclosporine and the prompt recurrence of proteinuria after stopping cyclosporine are compatible with this hypothesis.

Cyclosporine also had a pronounced effect on proteinuria in patients with membranous glomerulopathy. However its mode of action on the diseased glomerular barrier was different from that in minimal change disease. Before cyclosporine, in the untreated state, proteinuria was non-selective and the clearance of iothalamate, the fractional clearance of uncharged dextrans with a radius between 28-38 Å and filtration fraction were depressed significantly below values in our healthy volunteers. In contrast, the passage of dextrans with radius > 54 Å was increased. Applying Deen's theoretical model of glomerular solute transport on these findings, the abnormal dextran sieving can be explained by a loss of intrinsic ultrafiltration capacity (depressed K_f) and decreased barrier size selectivity. Using a ΔP of 40 mmHg we calculated K_f to be depressed threefold and the fraction of filtrate permeating the shunt pathway (ω_o) to be increased fivefold.

In contrast to the effects of cyclosporine on charge selectivity in patients with minimal change disease, the antiproteinuric action of cyclosporine in membranous glomerulopathy was the result of an increase in size selectivity of the glomerular filtration barrier. Blood pressure and GFR did not change significantly, although ERPF fell markedly by 35%. The fractional clearance of dextrans measuring 28-32 Å rose and that of large dextrans (50-58 Å) fell. Our computations revealed that the changes in glomerular sieving induced by CsA can be accounted for by a rise in K_f and a pronounced diminution of filtrate passing through the "shunt" pathway ω_o. Little or no effect on ω_o is predicted to occur when we varied ΔP throughout a wide range, between 24 and 52 mmHg (Fig. 3). Thus, the unavailability of data on ΔP in humans does not seriously influence our interpretations.

Theoretically, a CsA induced decrease in ΔP could also alter the structure of the glomerular basement membrane and thereby permeability characteristics [26]. Indeed, significant changes in permselectivity were observed in patients with glomerulopathy during treatment with drugs that decrease filtration pressure such

as indomethacin [11] and angiotensin converting-enzyme inhibitors [27]. These changes, however, were unvaryingly accompanied by a decrease in filtration fraction. In our Cyclosporine-treated patients filtration fraction rose, suggesting that glomerular hemodynamics per se cannot explain the antiproteinuric action of cyclosporine. We therefore conclude that non-hemodynamic factors, most likely of immunological origin, could be involved in the beneficial effects of CsA on proteinuria in membranous glomerulopathy.

In FSGS and MPGN, proteinuria was non-selective from the onset and remained so after treatment. No effect of cyclosporine on proteinuria was observed in these patients. Following CsA, renal hemodynamics were severely disturbed in FSGS and MPGN patients with both GFR and ERPF dropping markedly and renal vascular resistance rising. Interestingly, blood pressure also increased significantly in FSGS and MPGN whereas no change in blood pressure was observed in MCD and IMGN patients. This is in accordance with the previous report that patients who do not respond to treatment with CsA are more likely to be affected by its side effects [1]. It also argues against a hemodynamic mode of antiproteinuric action in the responding patients as the patients most severely affected by hemodynamic changes had no change in protein excretion.

If CsA is to be of clinical importance in nephrotic syndrome, it is essential that its effects are not solely hemodynamic in nature. If symptomatic reduction of proteinuria were the objective, the current therapeutic arsenal hold several drugs, such as indomethacin [28] and angiotensin converting-enzyme inhibitors [29], that probably decrease proteinuria through hemodynamic mechanisms but are less nephrotoxic with continued treatment. The results of the present study demonstrate that, in MCD and IMGN, low doses of CsA exert a dramatic effect on proteinuria with relatively minor effects on renal hemodynamics. Furthermore the decrease in renal function did not appear to be instrumental in reducing proteinuria. Therefore, in MG and MCD, the immunosuppressive action of CsA may play an important role in decreasing proteinuria.

In all patients in which CsA was discontinued proteinuria returned promptly. At present it is unclear wether a lasting remission can be obtained with longer treatment and several authors have suggested that prolonged treatment with low doses of CsA (2 to 4 mg/kg/day) is required to maintain remission [1,3]. However, it remains to be demonstrated that these lower doses are free of long-

term nephrotoxic side effects as evident morphological changes were observed at doses as low as 5 mg/kg/day in heart transplant recipients [30]. Therefore caution is in order for the long-term use of CsA in diseases with a relatively benign course, such as MCD and MG.

We conclude that in membranous glomerulopathy cyclosporine exerts its effects on proteinuria mainly through a change in the permeability characteristics of the glomerular basement membrane resulting in a decrease in shunt-flow, despite increased filtration fraction. In minimal change disease cyclosporine decreases proteinuria through an increased charge selectivity and appears to increase K_f.

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Chapter 5

Glomerular barrier function following conversion from cyclosporine to azathioprine in renal transplant recipients

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Abstract

The renal side-effects are the major limitation of the use of cyclosporine-A (CsA) in clinical transplantation. We studied the reversibility of changes in renal hemodynamics and glomerular barrier function in 17 patients with moderately impaired renal function at least one year after kidney transplantation. All patients were studied both during CsA treatment and three months after conversion to azathioprine (AZA). During AZA both glomerular filtration rate and effective renal plasma flow increased significantly (from 44.3±4.2 to 63.5±5.4 ml/min and from 192±12.8 to 260±14.6 ml/min respectively. Despite the marked changes in renal hemodynamics, no significant changes were observed in the fractional clearances of uncharged dextrans. When calculating the characteristics of the filtration barrier, we observed a trend towards an increase in the ultrafiltration coefficient (K_f). This trend was abolished when an increase in net filtration pressure (ΔP) was assumed to result from reduced pre-renal vasoconstriction. We conclude that, despite marked improvement of renal perfusion and glomerular filtration, conversion from CsA to AZA did not significantly alter the permeability characteristics of the glomerular filtration barrier in renal transplant recipients with moderately reduced renal function. Improvement in renal function following conversion could result from an increase

in either K_f or ΔP. Since renal plasma flow was increased significantly, the observed improvement in GFR is likely to be, at least in part, due to an increase in glomerular capillary plasma flow.

Introduction

Cyclosporine A (CsA) is an immunosuppressant drug used routinely in transplantation medicine today [1]. An important disadvantage of the use of CsA is its detrimental effect on renal function. Part of this effect is thought to be due to afferent renal vasoconstriction which is supposed to be reversible [2]. Also, CsA induces structural vascular abnormalities and interstitial changes resulting in striped fibrosis and glomerulosclerosis [3]. These changes lead to permanent loss of renal function. In order to prevent the onset of irreversible damage to the kidney we and others have attempted to convert renal transplant recipients receiving CsA to azathioprine (AZA) [4,5,6]. Although these studies have invariably demonstrated that renal function improves as a result of conversion, it appears that some renal dysfunction may persists even when converting subjects to AZA as early as 6 months after transplantation.

In heart transplant-recipients CsA has been shown to affect glomerular barrier function [7]. We have previously studied the effects of CsA on glomerular barrier function in patients with nephrotic syndrome [8]. In these patients CsA altered the glomerular permeability to dextrans by either increasing hydraulic permeability (minimal change disease) or improving the size-selective properties of the glomerular filtration barrier (membranous glomerulopathy). Although these effects are likely to be the result of remission of the initial disease process, a direct effect of CsA on the glomerular filtration barrier cannot be excluded.

In order to investigate the reversibility of renal dysfunction following renal transplantation, we studied the effect of conversion from CsA to AZA in patients with moderately impaired renal function (serum creatinine levels between 150 and 300 μmol/L). Apart from the measurement of renal plasma flow and GFR, we also determined the fractional clearances of neutral dextrans of graded size in order to study the effects of conversion on the glomerular filtration barrier. In

some of the patients a renal biopsy was performed at the start of the study, in order to exclude the presence of rejection.

Patients and methods

Study population

A total of 17 renal allograft recipients (14 male / 3 female; age 30 to 64 years) were included in this study. At entry all subjects had moderately impaired renal function (median serum creatinine 2.2 mg/dL, range 1.7 to 4.1 mg/dL). The median time after transplantation was 18 months (range 12 to 36 months). Patients with diabetes or proteinuria above 3 g/24 hr were excluded from the study. All subjects had received Cyclosporine A (CsA; whole blood trough levels between 150 and 300 pg/ml) and low-dose steroids from the time of transplantation onwards. Written informed consent was obtained in all patients and the ethical committee of the University Hospital Rotterdam-Dijkzigt had approved the protocol.

Study protocol

All patients were studied two times. After initial baseline clearance measurements, just prior to a renal biopsy, the patients were converted to azathioprine (AZA). During conversion the daily oral dose of prednisone was temporarily increased to 30 mg and then tapered by 5 mg per week to the initial dose of 10 mg. Three months after this conversion each subject underwent a final clearance study.

Clearance studies

In the morning the patients drank tap water 20 ml/kg body weight. Plastic cannulas were inserted into an antecubital vein of each arm. The infusion of dextrans and radiolabeled clearance markers was given in one arm, while blood samples were drawn from the other arm. Using a constant infusion technique

GFR and ERPF were determined, by measuring the clearances of ^{125}I-iodothalamate and ^{131}I-orthoiodohippurate (Amersham International, Amersham, Bucks, U.K.), respectively. The average priming doses were 4.4 and 8.8 μCi, and sustaining infusion rates were 1 and 8 μCi/h respectively. Both were adjusted for renal function using a method described previously [9]. All patients remained in the supine position during the study period. However, they were allowed to stand in order to void urine. After a 1 hour equilibration period, the bladder was emptied by voiding. After a slow injection of 200 mg of dextran-10 (Promiten; NPBI, Emmercompascuum, the Netherlands) in order to avoid anaphylactic reactions, a 5 minute infusion of 240 mg of dextran-70 (40 ml; Macrodex; NPBI) and a continuous infusion of 20 mg per minute dextran-40 (Rheomacrodex; NPBI) were administered. During three carefully timed 30 minutes intervals urine was collected. From these three clearance periods the mean GFR and ERPF were calculated. To calculate the clearance values the standard formula was used. Fractional clearances of dextrans were calculated using the equation:
Fractional dextran clearance = $[(U/P)_{DEXTRAN} / (U/P)_{^{125}I\text{-}IODOTHALAMATE}]$
in which U and P denote the urinary and plasma concentrations of dextran and ^{125}I-iodothalamate.

Laboratory procedures

CsA trough levels were measured in whole blood using a specific radioimmunoassay (Cyclotrac, Incstar, Stillwater, USA). Creatinine levels in serum and urine were measured using a colorimetric assay. Serum and urine total protein concentrations were measured using a modified biurete method whereas albumin was measured in a immunoturbidimetric assay. For the measurement of effective renal plasma flow and GFR ^{125}I and ^{131}I were counted in a gamma-scintillation counter.

Dextran was assayed in protein-free filtrates of plasma and urine (trichloric acetic acid 40%). These filtrates were separated into narrow fractions by gel-permeation chromatography using the method described by Granath et al. [10]. A sephacryl S-300 column (Pharmacia, Upsala, Sweden) of 180 ml bed-volume and 90 cm in length was used. The eluent was a 0.01 M Tris buffer at pH 7.0 with 0.15 M NaCl containing 1 mM EDTA. Blue dextran (Mw 2,000,000 D) was used to

determine void volume (V_o), and the column was calibrated using dextran T10, T40 and T70 (Pharmacia). The fractional volume available to the solute (K_{AV}) was then calculated from:

$$K_{AV} = (V_e - V_o)/(V_t - V_o)$$

where V_e is the volume necessary to displace a solute from the column, and V_t is the total bed-volume of the gel column. Effective molecular radii for the individual dextran fractions were calculated from K_{AV}. After gel permeation chromatography, eluted fractions were assayed for dextran using the anthrone method of Scott and Melvin [11].

Analysis of glomerular barrier function

To analyze the size-selective properties of the glomerular barrier, we used a heteroporous model of the glomerular capillary wall, as described by Deen et al [12]. This model has been shown to provide the most satisfactory representation of dextran sieving. In this model the major portion of the capillary wall is perforated by restrictive cylindrical pores of identical radius (r_0). In addition, this model assumes a parallel 'shunt pathway' that does not discriminate on the basis of dextran size, and through which a small fraction (ω_0) of the filtrate volume passes. The size of this fraction is not merely dependent on the properties of the capillary wall, but also on intracapillary oncotic pressure. Therefore a quantity is introduced that is closely related to ω, but that is independent of oncotic pressure and therefore characteristic of the membrane per se. This quantity (ω_0) is the fraction of the volume flux that would pass through the shunts if plasma proteins were absent. The membrane barrier to dextrans is fully characterised by r_0, ω_0 and K_f, where K_f is the product of effective hydraulic permeability and glomerular capillary surface area.

Histological evaluation

All biopsy material was subjected to light microscopic and immunofluorescence investigations. Cyclosporine nephrotoxicity was defined by the presence of CsA associated arteriolopathy (i.e. either lumpy protein deposits in the arteriolar wall or mucoid, paucicellular thickening of the intima leading to narrowing of the

arteriolar lumen), striped fibrosis or both [13].

Statistical analyses

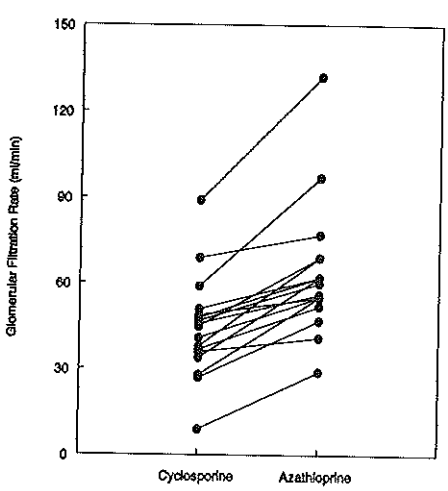
Data before and after conversion from CsA to AZA were compared using the Student t-test for paired data. All data is presented as mean ± SEM.

Results

Seventeen patients were included into the study and all patients completed the protocol. Renal function was moderately impaired as the mean GFR at baseline was 44.3±4.2 ml/min (Table 1).

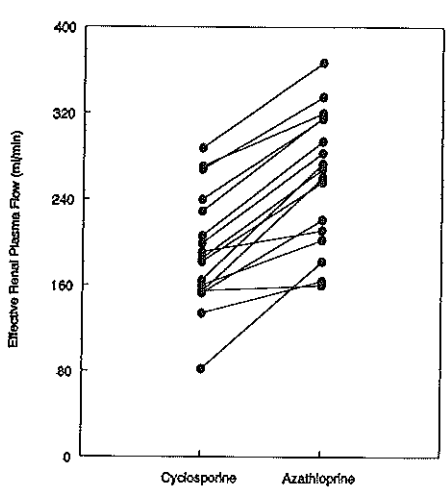
Three months after conversion from CsA to AZA renal function was improved significantly. Serum creatinine levels decreased from 209±11.3 to 152±4.9 μmol/L ($p < 0.01$). The estimated creatinine clearance, calculated from the

Fig. 1



Marked increase in GFR following conversion from CsA to AZA in 17 renal transplant recipients.

Fig. 2



Marked increase in effective renal plasma flow following conversion from CsA to AZA in 17 renal transplant recipients.

Table 1

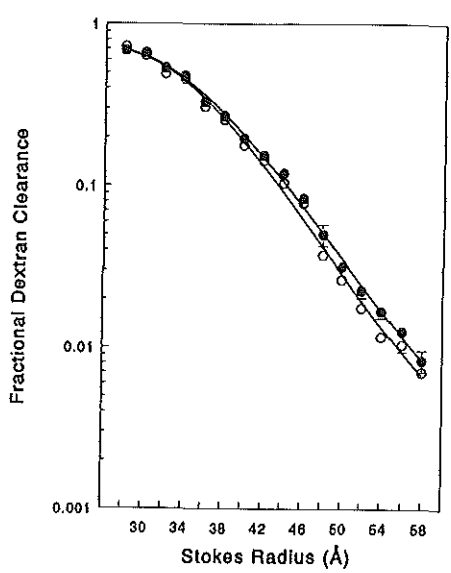
Renal Hemodynamics During Cyclosporine and 3 Months After Conversion to Azathioprine

	Cyclosporine	Azathioprine	Probability Value
GFR (mL/min)	44.3 ± 4.2	63.5 ± 5.4	<0.01
Effective renal plasma flow (mL/min)	192 ± 12.8	260 ± 14.6	<0.01
Filtration fraction	0.23 ± 0.01	0.24 ± 0.01	NS

equation of Cockcroft and Gault [14] increased from 43.9±3.8 to 58.3±3.8 ml/min. This estimation of GFR proved to be accurate as GFR measured by means of the clearance of iothalamate increased from 44.3±4.2 to 63.5±5.4 ml/min after conversion (Fig 1). Renal perfusion also increased after cessation of CsA treatment as the ERPF increased from 192±12.8 to 260±14.6 ml/min (Fig 2). No change in filtration fraction occurred.

Despite these marked changes in renal hemodynamics, the fractional clearances of polydisperse neutral dextrans after conversion were not different from baseline (Fig 3). Calculated basement membrane parameters were not significantly altered by conversion to AZA (Table 2). Urinary albumin excretion rates were not

Fig. 3



No significant effect on the fractional clearances of graded dextrans during CsA (open circles) and 3 months after conversion to AZA (filled circles).

Table 2

	Basement Membrane Characteristics		
	K_f	r_0	ω_0
Cyclosporine			
ΔP 35 mm Hg			
E_{hlp} 0.6	8.2	55.8	1.5
E_{hlp} 0.7	8.3	55.7	1.4
Azathioprine			
ΔP 35 mm Hg			
E_{hlp} 0.6	10.9	55.5	2.0
E_{hlp} 0.7	11.3	55.8	2.0
ΔP 40 mm Hg			
E_{hlp} 0.6	7.4	55.5	2.6
E_{hlp} 0.7	7.5	55.5	2.6

significantly altered by conversion (232±59 mg/24 h during CsA vs. 280±121 mg/24 h after 3 months of AZA; n.s.). The response in dextran clearance did not differ with albuminuria levels (urinary albumin excretion >1000 mg/day vs. <150 mg/day).

Before conversion, renal biopsies were performed in 13 out of 18 patients. Histological evidence of cyclosporine nephrotoxicity was found in five patients. The response of renal hemodynamics to conversion was not different in these subjects compared to those in which CsA toxicity was not deemed present.

Two episodes of acute rejection were observed after conversion to AZA. In both instances renal dysfunction was rapidly reversed by 1000 mg of methylprednisolone on three consecutive days. Liver function and haematological parameters were not influenced by 3 months of treatment with AZA.

Discussion

Previous studies have demonstrated that in renal transplant recipients, cyclosporine nephrotoxicity is partly reversible after conversion to azathioprine [4,5]. Histological evidence, obtained from repeat biopsies, also indicates that

cyclosporine-induced changes may recede after discontinuation of CsA treatment [15].

The present study demonstrates that in renal transplant recipients with moderately impaired renal function, more than one years post-transplant, improvement in the functional impairment of the kidney can be obtained. Both renal plasma flow and glomerular filtration rate increased by approximately 40%. Beside a reduction in GFR, treatment with CsA has been associated with alterations in the glomerular barrier function. Myers et al. compared the glomerular permeability characteristics in heart transplant recipients treated with either CsA or AZA from the time of transplantation onwards [7]. They demonstrated a trend towards decreased fractional clearance of small dextran molecules which could point towards a reduction of the ultrafiltration coefficient K_f. Until now, no data exist concerning the reversibility of such changes.

Our data indicate that, despite marked improvement in renal function, no significant change in dextrans clearances could be observed. This is surprising as the theory of passage through restrictive pores predicts that the fractional clearance of dextrans should have decreased as a result of increased renal perfusion. As flow increases, the average concentration gradient of the solute decreases thereby decreasing its fractional clearance [16]. When calculating the permeability characteristics of the glomerular filtration barrier, which are independent of changes in the determinants of GFR we did observe a trend towards an increase in K_f. This trend did not reach statistical significance. Essential to the calculation of such parameters is the net hydraulic filtration pressure (ΔP). Unfortunately, ΔP cannot be measured in humans. However, both animal studies by Barros et al. [17] and physiological studies in heart transplant recipients [7] suggest that ΔP is depressed during cyclosporine therapy. This may be the consequence of afferent arteriolar vasoconstriction [18]. Dextran profiles performed by the Stanford group suggest that ΔP is close to 35 mmHg in CsA treated patients and approximately 40 mmHg during AZA treatment [7]. Thus, after conversion to AZA, ΔP could be increased due to reduced pre-renal vasoconstriction. Assuming an increase in ΔP to 40 mmHg, subsequent calculation of the GBM parameters indicates that the trend towards increased K_f would be annihilated (Table 2).

A confounding factor in the interpretation of the dextran sieving data is the

possible variation of the renal extraction of hippurate following conversion. Previous studies have suggested that CsA may reduce E_{hip} [19]. If conversion were to increase E_{hip}, the change in renal plasma flow may be overestimated. However, small increases in E_{hip} are predicted to have a negligible effect on basement membrane parameters (Table 2) [20].

Glomerular size-selectivity, as represented by ω_0, was not significantly altered by cessation of CsA treatment. We previously demonstrated that in nephrotic syndrome due to minimal change disease and membranous glomerulopathy treatment with CsA restores permselectivity [8]. The present data therefore supports the notion that the response to CsA in patients with a nephrotic syndrome is due to an immunological effect rather than a direct effect on the filtration barrier.

We conclude that, despite marked improvement of renal perfusion and glomerular filtration, conversion from CsA to AZA did not significantly alter the permeability characteristics of the glomerular filtration barrier in renal transplant recipients with moderately reduced renal function. Improvement in renal function following conversion could result from an increase in either K_f or ΔP. Since renal plasma flow was increased significantly, the observed improvement in GFR is likely to be, at least in part, due to an increase in glomerular capillary plasma flow.

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Chapter 6

Effect of synthetic human atrial natriuretic peptide (102-126) in nephrotic syndrome

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Kidney International 1988;34:717-724.

Abstract

Synthetic human ANP (102-126) or vehicle was intravenously administered to eight patients with non-edematous nephrotic syndrome. ANP was given in ascending doses, each dose for one hour, 2-3 days apart. Four patients received 0.03, 0.10 and 0.45 ug/kg/min of ANP, and four received 0.015, 0.06 and 0.20 ug/kg/min. Natriuresis increased at all doses; by 179±13.6 % (mean±SEM) ($p<0.05$) at 0.015 ug/kg/min and by 660±71.5 % ($p<0.01$) at 0.20 ug/kg/min. Urinary albumin excretion increased by 138±30.1 % ($p<0.05$) at 0.015 ug/kg/min of ANP and by 534±132 % ($p<0.01$) at 0.20 ug/kg/min. Immunoglobulin G excretion increased proportionally to albumin excretion. Hematocrit and serum albumin concentration increased after ANP. In each patient the percent reduction of plasma volume calculated from the effect on serum albumin was smaller than the hemoconcentration calculated from the effect on hematocrit, suggesting a loss of albumin from the intravascular compartment. This could not be accounted for by the increased glomerular filtration of albumin. Blood pressure and effective renal plasma flow decreased and filtration fraction increased after ANP. Plasma renin was suppressed at lower doses of ANP but was stimulated, together with plasma noradrenaline, at higher doses. Basal plasma ANP levels were normal and rose at the lowest dose to levels slightly

above the physiological range.
Thus, ANP appears to increase the transcapillary filtration of intravascular protein in the glomerulus and possibly elsewhere in the body. In non-edematous patients with nephrotic syndrome the proteinuric and natriuric effects of ANP were already seen at low doses. Endogenous ANP may therefore have similar effects in these patients.

Introduction

Mammalian atria contain peptides with potent natriuretic, diuretic and vasorelaxant properties [1]. A series of these peptides, derived from a larger precursor molecule, have been isolated. In man a single peptide, human Atrial Natriuretic Peptide (99-126), which consists of the 28 aminoacids of the carboxy-terminal of its precursor molecule, appears to be the main circulating form [2]. The effects of synthetic ANP preparations on urinary sodium and water excretion and on systemic and renal hemodynamics have been studied in healthy volunteers and in patients with essential hypertension, congestive heart failure and liver cirrhosis [3,4,5].
This paper reports on the effects of incremental doses of synthetic human ANP (102-126) on urinary excretion of sodium and water in patients with nephrotic syndrome. Since it is not known whether ANP has an effect on urinary protein excretion, we also report on the changes in urinary albumin and immunoglobulin G following ANP administration in this particular group of patients. The tubular capacity of protein reabsorption is saturated in nephrotic syndrome so that changes in urinary protein excretion are a measure of changes in filtered protein [6]. In addition, we measured the effects of ANP on hematocrit, serum albumin, blood pressure, glomerular filtration rate and renal plasma flow as well as on plasma renin and noradrenaline.

Methods

Study protocol

The study was performed in eight male subjects with nephrotic syndrome (proteinuria 4.0 to 9.0 g/24 hr) with only moderate impairment of renal function (Table 1). There was no evidence of any other than renal disease. Following a two week run-in period, in which all medication was discontinued, 24 hr urinary sodium excretion was measured on two consecutive days. Daily sodium intake was then adjusted to the average 24 hr sodium excretion at the end of the run-in period. When sodium balance was achieved (24 hr urinary sodium excretion within 15 percent of daily sodium intake), the patient was admitted to the hospital. Infusions of placebo and three incremental doses of ANP were given, each after a 2-3 day interval. Four patients (group A) received human ANP (102-126) at a rate of 0.03, 0.10 and 0.45 ug/kg/min. Because significant effects on urinary sodium and protein excretion were already seen at doses as low as 0.03 and 0.10 ug/kg/min, we studied a second group of four patients (group B) at a dose rate of 0.015, 0.06 and 0.20 ug/kg/min.

After a light breakfast patients drank tap water, 20 ml/kg body weight in 20 min. Plastic cannulas were then inserted into an antecubital vein of each arm. One arm was used for infusion of ANP, Thalamate and Hippuran, while blood samples were drawn from the other arm. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were determined by a constant infusion technique, by measuring the renal clearances of ^{125}I-Thalamate and ^{131}I-Hippuran (Amersham, UK) respectively. The priming dose was 0.08 to 0.1 uCi/kg for Thalamate, and 0.3 to 0.4 uCi/kg for Hippuran. The sustaining infusion rates were 0.05 and 0.2

Table 1

Clinical patient data

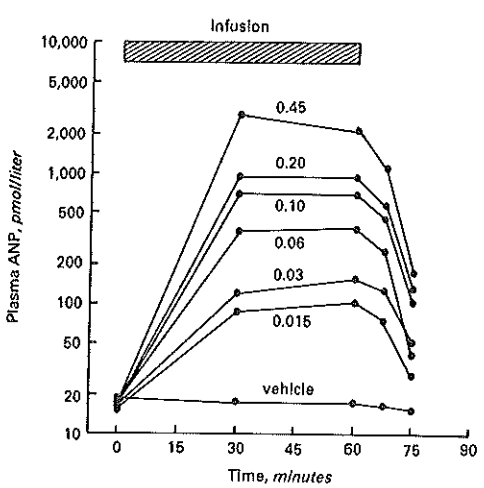
Patient group	Diagnosis	Age *yr*	Body weight *kg*	Basal urinary protein excretion *g/24 hr*	Serum creatinine *μmol/liter*	Basal urinary sodium excretion *mmol/24 hr*
A	Membranous glomerulonephritis	36	91.5	4.0	86	123
A	Alport's syndrome	25	80.5	4.5	301	192
A	Hereditary glomerulonephritis	22	58.5	8.0	148	136
A	Membranous glomerulonephritis	19	70.5	8.0	94	170
B	Focal segmental glomerulosclerosis	35	69.3	6.0	105	70
B	Membranoproliferative glomerulonephritis	55	71.5	7.5	202	135
B	Minimal change disease	45	67.7	7.5	83	125
B	Alport's syndrome	22	70.5	9.0	116	176

uCi/min respectively [7].

Patients remained in the supine position but were allowed to stand in order to pass urine. After the water load, urine was collected at 20 min intervals and blood samples were taken at the midpoint of each collection period. A steady-state (two consecutive urine collections varying less then 1 ml/min) was achieved after approximately 120 min. The average of the two last urine collections served as baseline. After each urine collection the urine volume was measured and that volume plus 1 ml/min (insensible loss) was replaced orally. Following the baseline period human ANP (102-126) (Wy 47.663, Wyeth Research Laboratories, Philadelphia, Pennsylvania, USA) in vehicle (0.005 M acetic acid in glucose 5 % containing 100 mg/ml mannitol) or placebo (vehicle) were infused intravenously for 60 min. Following the infusion the collection of urine and blood samples was continued for 120 min. The amount of sodium excreted above baseline during each experiment (infusion period and the 120 min thereafter) was replaced in divided doses by giving sodium chloride capsules, two capsules a day, on the days between the experiments.

Blood pressure and heart rate were measured at 10 min intervals before and after the infusion of ANP or vehicle and at 2 min intervals during the infusions.

Fig. 1



Plasma ANP levels before, during and after infusion of human ANP (102-126) or vehicle in patients with nephrotic syndrome. Doses are given in μg/kg/min.

Hematocrit and the plasma concentrations of renin and noradrenaline were measured before and after 60 min of infusion of ANP or vehicle, and at the end of the experiment 120 min after the infusion had been stopped. The serum and urinary concentrations of albumin, sodium, ^{131}I-Thalamate and ^{125}I-Hippuran were measured in each 20 min clearance period.
The experimental protocol was approved by the hospital ethical committee and informed consent was obtained from the patients participating in this study.

Analytical methods

Blood pressure was determined with an oscillometric device (Accutorr, Datascope Corp, Paramus, New Jersey, USA). The means of three consecutive readings were used for analysis.
Serum albumin concentration was measured by means of the bromocresol green binding method on the Technicon SMAC [8]. Urinary albumin and immunoglobulin G (IgG) concentrations were measured by single radial immunodiffusion using the method originally described by Mancini [9]. The between-run coefficients of variation were 1.7 % for the bromocresol green method and 5.0 % for the radial immunodiffusion.
The plasma concentration of active renin was measured by radioimmunoassay [10]. Plasma noradrenaline was determined by high pressure liquid chromatography with electrochemical detection [11]. Plasma and urinary ^{125}I and ^{131}I activity were measured in a gamma scintillation counter.
Plasma ANP levels were measured by dr J.Nussberger (Centre Hospitalier Vaudois, Lausanne, Switzerland), after extraction, by radioimmunoassay using an antibody directed against human ANP (99-126) [12]. This antibody shows 100 % cross-reactivity with human ANP (102-126). The normal value in 40 supine healthy volunteers is 17.9 $\pm$8.1 pmol/L (mean $\pm$ SD).

Calculations

When we assume the red-cell volume to be constant during infusion of ANP, the change in plasma volume (PV) at time t after the start of the infusion can be calculated from the changes in hematocrit (Hct) using the following formula:

$$\frac{PV_t}{PV_0} = \frac{1/(1-Hct_0)-1}{1/(1-Hct_t)-1} \qquad \text{(Eq 1)}$$

When the total amount of albumin within the vascular compartment remains unaltered, the change in plasma volume at time t can also be calculated from the changes in serum albumin (Alb) concentration:

$$\frac{PV_t}{PV_0} = \frac{Alb_0}{Alb_t} \qquad \text{(Eq 2)}$$

Statistical analysis

Data are presented as means ± SEM. The effects of ANP on the renal excretion of water and solutes, during the 60 min infusion period and 60 minutes thereafter, were assessed by multiple regression analysis, in which ANP was compared to vehicle. All other data were analyzed by means of one way analysis of variance. When a significant difference was demonstrated, the levels at the end of the 60 min infusion and 120 min thereafter were compared with baseline by means of the Student-Neuman-Keuls test.

Results

The infusions were carried out without complications and data collection was complete. Results are summarized in Tables 2, 3 and 4. Three subjects experienced a feeling of lightheadedness when standing to urinate, in each case during the last 20 min of infusion at the time of the third infusion (0.06 or 0.10 ug/kg/min).

Basal plasma ANP concentration was normal, 19.2±3.6 pmol/L. During infusion of human ANP (102-126) plasma levels of ANP increased at all doses (Fig 1). Peak levels of ANP were 103±16 pmol/L ($p<0.01$) at an infusion rate of 0.015

Table 2

Effects of human ANP (102-126)

Dose	Plasma ANP *pmol/liter*	Mean blood pressure *mm Hg*	Heart rate *bpm*	Hematocrit *liter/liter*	Serum albumin *g/liter*	Plasma renin *µU/ml*	Plasma noradrenaline *pmol/ml*
Group A							
Vehicle							
0 min	18.9 ± 1.3	105 ± 3.3	67.1 ± 3.8	0.46 ± 0.02	29.4 ± 1.5	20.2 ± 3.0	1.50 ± 0.18
60 min	17.8 ± 1.5	101 ± 3.4	64.6 ± 3.5	0.45 ± 0.02	29.6 ± 1.6	19.7 ± 2.5	1.54 ± 0.11
180 min		102 ± 3.2	63.9 ± 4.1	0.46 ± 0.02	29.1 ± 1.5	18.9 ± 3.3	1.59 ± 0.08
ANP *0.03 µg/kg/min*							
0 min	16.3 ± 3.1	98.0 ± 6.2	64.3 ± 4.0	0.45 ± 0.01	28.9 ± 1.9	31.8 ± 6.9	1.48 ± 0.14
60 min	154 ± 14[b]	89.5 ± 6.4[a]	65.4 ± 4.0	0.47 ± 0.01	29.0 ± 2.1	17.7 ± 3.8[a]	1.89 ± 0.13
180 min		91.8 ± 5.0	61.6 ± 3.5	0.45 ± 0.01	28.3 ± 2.3	31.9 ± 3.7	1.56 ± 0.11
ANP *0.10 µg/kg/min*							
0 min	18.8 ± 3.6	98.2 ± 4.6	65.3 ± 3.2	0.43 ± 0.01	29.0 ± 1.5	47.8 ± 7.5	1.69 ± 0.17
60 min	721 ± 100[b]	80.6 ± 3.8[b]	68.7 ± 4.9	0.46 ± 0.01[a]	31.5 ± 2.3[a]	43.1 ± 8.4	2.22 ± 0.13[b]
180 min		88.5 ± 5.1[a]	64.6 ± 2.1	0.43 ± 0.01	27.3 ± 1.6	43.0 ± 6.9	1.60 ± 0.04
ANP *0.45 µg/kg/min*							
0 min	19.8 ± 3.9	98.8 ± 4.7	60.5 ± 4.6	0.40 ± 0.01	29.2 ± 1.3	38.1 ± 7.4	1.57 ± 0.11
60 min	2170 ± 410[b]	80.5 ± 5.0[b]	73.7 ± 7.2[a]	0.44 ± 0.01[a]	32.8 ± 2.2[a]	96.6 ± 9.5[a]	3.22 ± 0.10[b]
180 min		87.5 ± 3.0[a]	68.1 ± 7.3	0.41 ± 0.01	28.0 ± 1.8	47.7 ± 6.7	1.95 ± 0.11
Group B							
Vehicle							
0 min	21.3 ± 1.0	102 ± 3.1	69.1 ± 3.7	0.38 ± 0.02	29.2 ± 1.3	17.9 ± 3.1	1.64 ± 0.19
60 min	20.0 ± 1.4	101 ± 3.0	64.6 ± 3.5	0.38 ± 0.02	29.6 ± 1.6	18.2 ± 2.5	1.59 ± 0.11
180 min		100 ± 3.4	64.6 ± 4.1	0.38 ± 0.02	29.5 ± 1.4	18.1 ± 3.3	1.70 ± 0.10
ANP *0.015 µg/kg/min*							
0 min	14.3 ± 2.7	104 ± 2.4	69.1 ± 6.7	0.37 ± 0.02	31.2 ± 1.7	34.6 ± 7.8	1.79 ± 0.23
60 min	103 ± 16[b]	98.5 ± 2.7	69.5 ± 5.4	0.38 ± 0.03	32.0 ± 1.6	25.4 ± 5.6	2.08 ± 0.19
180 min		99.9 ± 2.2	65.9 ± 6.5	0.37 ± 0.02	30.0 ± 1.6	23.7 ± 3.4	2.04 ± 0.16
ANP *0.05 µg/kg/min*							
0 min	19.3 ± 4.3	103 ± 5.5	66.2 ± 5.7	0.34 ± 0.02	30.9 ± 1.3	26.8 ± 1.6	1.57 ± 0.07
60 min	381 ± 71[b]	91.6 ± 4.1[a]	68.8 ± 6.7	0.36 ± 0.02	32.3 ± 1.3	34.9 ± 5.4	1.75 ± 0.16
180 min		101 ± 4.5	65.1 ± 4.9	0.34 ± 0.02	29.8 ± 1.0	32.9 ± 5.2	1.59 ± 0.08
ANP *0.20 µg/kg/min*							
0 min	19.5 ± 3.3	99.4 ± 3.5	69.8 ± 4.2	0.30 ± 0.02	31.5 ± 0.2	26.3 ± 3.5	1.17 ± 0.07
60 min	939 ± 80[b]	90.2 ± 1.4[a]	70.8 ± 5.8	0.35 ± 0.03[a]	33.5 ± 0.6[a]	31.9 ± 5.0	1.73 ± 0.12[a]
180 min		95.8 ± 2.3	65.3 ± 4.6	0.31 ± 0.02	31.3 ± 0.4	27.9 ± 2.4	1.36 ± 0.12

Values are means ± SEM ($N = 4$).
[a] $P < 0.05$ versus time 0 min
[b] $P < 0.01$ versus time 0 min

ug/kg/min and 2170±410 pmol/L ($p<0.01$) at 0.45 ug/kg/min (Table 2). ANP returned to baseline in about 15 min after the infusion had been stopped.

Sodium excretion increased at each dose of ANP (Fig 2, Table 4); it increased by 179±13.6 % ($p<0.05$) at 0.015 ug/kg/min of ANP and by 660±71.5 % ($p<0.01$) at 0.20 ug/kg/min. Urine production also increased significantly at all doses of ANP, except at the lowest dose in group A (0.03 ug/kg/min).

During ANP infusion urinary albumin excretion increased at each dose (Fig 3, Table 4); it increased by 138±15.1 % ($p<0.05$) at 0.015 ug/kg/min of ANP and by 534±66.2 % ($p<0.01$) at 0.20 ug/kg/min. Changes in IgG excretion were

Table 3

Effects of 60 min infusion of human ANP (102-126)

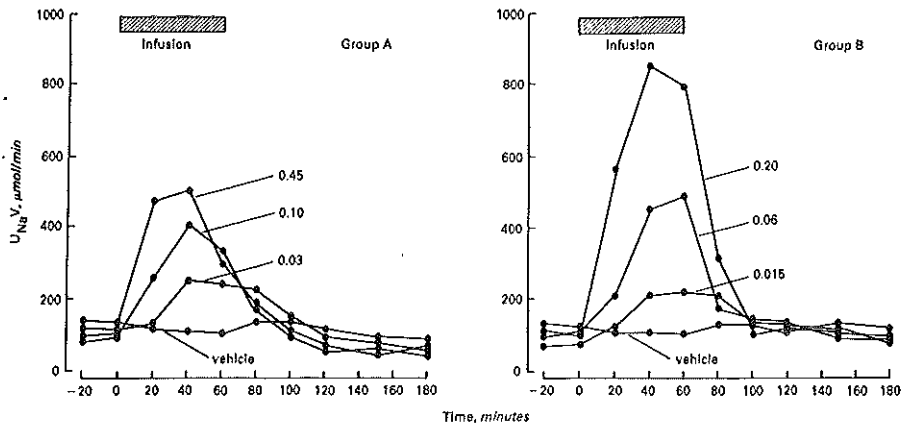
Dose	Albumin excretion *mg/min*	Fractional excretion of albumin %	GFR *ml/min*	ERPF *ml/min*	Filtration fraction
Group A					
Vehicle					
0 min	2.2 ± 1.0	0.09 ± 0.02	100 ± 9.7	460 ± 75.3	0.23 ± 0.02
60 min	2.1 ± 1.8	0.11 ± 0.03	101 ± 11	456 ± 72.9	0.24 ± 0.01
180 min	1.1 ± 0.8	0.07 ± 0.04	100 ± 11	452 ± 69.9	0.24 ± 0.02
ANP *0.03 µg/kg/min*					
0 min	2.1 ± 1.0	0.08 ± 0.04	95.4 ± 7.8	466 ± 50.5	0.22 ± 0.02
60 min	6.8 ± 6.2[a]	0.30 ± 0.05[a]	99.6 ± 7.9	406 ± 32.4	0.25 ± 0.01[a]
180 min	1.4 ± 3.8	0.06 ± 0.02	108 ± 8.7	508 ± 27.8	0.23 ± 0.01
ANP *0.10 µg/kg/min*					
0 min	1.6 ± 1.2	0.07 ± 0.04	103 ± 6.7	527 ± 48.5	0.22 ± 0.02
60 min	5.9 ± 3.8[a]	0.90 ± 0.06[b]	107 ± 9.7	441 ± 49.9[a]	0.25 ± 0.01[a]
180 min	1.0 ± 0.8	0.05 ± 0.02	111 ± 10	468 ± 52.1	0.25 ± 0.01
ANP *0.45 µg/kg/min*					
0 min	1.7 ± 1.2	0.12 ± 0.03	106 ± 8.4	544 ± 83.0	0.21 ± 0.01
60 min	12 ± 6.8[b]	0.38 ± 0.10[a]	104 ± 8.4	415 ± 44.9[a]	0.31 ± 0.02[b]
180 min	1.4 ± 1.0	0.06 ± 0.01	110 ± 11	434 ± 52.3	0.26 ± 0.01
Group B					
Vehicle					
0 min	4.1 ± 1.6	0.11 ± 0.02	98.9 ± 9.6	445 ± 74.4	0.22 ± 0.01
60 min	4.4 ± 2.4	0.13 ± 0.03	99.3 ± 10	442 ± 73.7	0.23 ± 0.02
180 min	2.3 ± 0.8	0.09 ± 0.04	100 ± 12	440 ± 68.1	0.22 ± 0.02
ANP *0.015 µg/kg/min*					
0 min	3.7 ± 2.8	0.11 ± 0.04	87.4 ± 7.7	445 ± 68.2	0.22 ± 0.01
60 min	8.8 ± 2.2[a]	0.29 ± 0.05[a]	86.8 ± 8.9	370 ± 47.6	0.24 ± 0.01
180 min	3.7 ± 2.2	0.13 ± 0.02	85.3 ± 7.9	394 ± 54.6	0.25 ± 0.01
ANP *0.06 µg/kg/min*					
0 min	4.2 ± 2.0	0.14 ± 0.04	95.4 ± 8.8	522 ± 102	0.21 ± 0.02
60 min	12.0 ± 3.6[a]	0.35 ± 0.06[a]	93.7 ± 10	403 ± 62.2[a]	0.25 ± 0.02[a]
180 min	3.9 ± 0.8	0.13 ± 0.02	89.5 ± 11	423 ± 79.2	0.23 ± 0.01
ANP *0.20 µg/kg/min*					
0 min	3.1 ± 0.6	0.11 ± 0.03	85.5 ± 8.1	456 ± 77.4	0.21 ± 0.02
60 min	19.7 ± 15[b]	0.45 ± 0.10[a]	88.3 ± 9.7	433 ± 74.6	0.23 ± 0.02
180 min	3.5 ± 1.8	0.1 ± 0.01	83.5 ± 12	421 ± 87.1	0.22 ± 0.02

Values are means ± SEM ($N = 4$).
[a] $P < 0.05$ versus time 0 min
[b] $P < 0.01$ versus time 0 min

parallel to those in albumin excretion (Fig 3, Table 4). IgG excretion increased by 83.3±16.5 % ($p<0.05$) at 0.015 ug/kg/min of ANP and by 300±25.1 % ($p<0.01$) at 0.20 ug/kg/min. The change in total albumin excretion was proportional to the change in fractional excretion (albumin clearance / creatinine clearance)(Table 3).

Infusion of ANP had no significant effect on GFR but ERPF decreased significantly at infusion rates of 0.06, 0.10, and 0.20 ug/kg/min (Table 3). Filtration fraction (GFR / ERPF) increased by 19.0±6.1 % ($p<0.05$) at 0.06 ug/kg/min of ANP and by 47.6±12 % ($p<0.01$) at 0.20 ug/kg/min.

Fig. 2



Effect of human ANP (102-126) or vehicle on urinary excretion of sodium in patients with nephrotic syndrome (groups A and B). Doses are given in μg/kg/min.

Hematocrit increased at 0.10, 0.20 and 0.45 ug/kg/min of ANP (Table 2). Serum albumin also increased at these doses of ANP.

Blood pressure fell during infusion of ANP at all doses (Table 2), except the lowest (0.015 ug/kg/min). At high doses the effect on blood pressure lasted throughout the 120 min period following infusion. Heart rate rose only at the highest dose of ANP (0.45 ug/kg/min)(Table 2).

Plasma renin was suppressed at 0.03 ug/kg/min of ANP (Table 2). Renin did not change after infusion of 0.06 to 0.20 ug/kg/min, and it rose after 0.45 ug/kg/min of ANP. Plasma noradrenaline rose during infusion of 0.10, 0.20 and 0.45 ug/kg/min of ANP (Table 2).

Discussion

Basal ANP levels were normal in our nephrotic subjects. This has also been observed by others [13]. During infusion of ANP at a rate of 0.015 or 0.03 ug/kg/min, plasma ANP rose to values within the pathophysiological range. At higher doses of ANP the plasma levels reached pharmacological rather than

Fig. 3

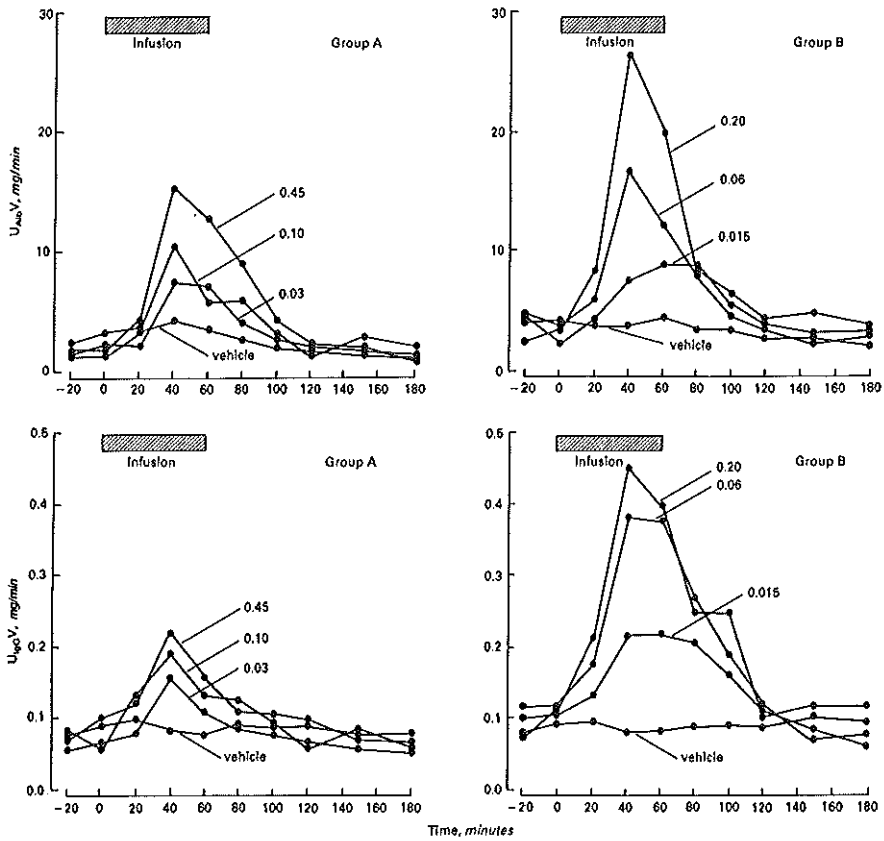


Fig. 3. *Effect of human ANP (102-126) or vehicle on urinary excretion of albumin and IgG in patients with nephrotic syndrome (groups A and B).* Doses are given in μg/kg/min.

physiological values.

Infusion of ANP induced natriuresis and diuresis and lowered blood pressure in our patients. These effects have also been observed in other disease states as well as in healthy subjects [1]. The published results in normal subjects cannot be readily compared to our study because of differences in sodium intake and baseline urine flow [4,14]. With these restrictions taken into account, the increase in sodium and water excretion in our nephrotic subjects appears to be at least equal to that in normal subjects. It is perhaps surprising that the effect of ANP

Table 4

Increase in urinary flow and sodium and protein excretion following infusion of human ANP (102-126)

Dose	Urine	Sodium	Albumin	IgG
μg/kg/min	*ml*	*mmol*	*mg*	
Group A				
0.03	105	9.15[a]	165[a]	2.7[a]
0.10	235[a]	15.3[a]	240[a]	4.6[a]
0.45	195[a]	21.6[a]	490[a]	5.2[a]
Group B				
0.015	230[a]	5.10[a]	260[a]	7.5[a]
0.06	280[a]	19.5[a]	510[a]	16[a]
0.20	450[b]	36.2[b]	940[b]	21[b]

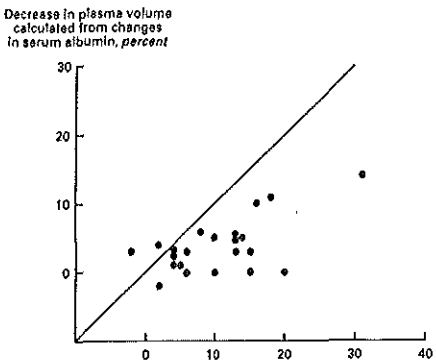
Values are means ($N = 4$).
[a] $P < 0.05$ versus vehicle
[b] $P < 0.01$ versus vehicle

on sodium and water excretion in nephrotics is similar to that in normal subjects, since the nephrotic syndrome is often associated with sodium and water retention [15] and the response to ANP is usually blunted in patients with heart failure or liver cirrhosis and edema. An attenuation of the natriuretic effect of ANP has also been reported in nephrotic syndrome, in rats [16] as well as in humans [17]. Our patients were not edematous when on normal sodium diet. We therefore doubt whether the results of this study can be extrapolated to patients with nephrotic syndrome and edema. The effects of ANP appeared to be blunted in group A as compared to group B. This could be the result, at least in part, of a somewhat lower daily sodium intake in group A (126 versus 155 mmol/24 hr, Table 1), as this attenuates the renal responsiveness to ANP [14].

Infusion of ANP is known to alter renal hemodynamics. In normal subjects glomerular filtration rate either increases or is not altered by ANP and effective renal plasma flow is lowered [4,14]. A consistent finding in previous studies is an increase in glomerular filtration fraction, calculated as the quotient of glomerular filtration rate and effective renal plasma flow. In our patients glomerular filtration rate did not change, effective renal plasma flow decreased and filtration fraction rose during infusion of ANP. As the renal extraction of Hippuran was not measured, an effect of ANP on proximal tubular excretion of Hippuran cannot be excluded.

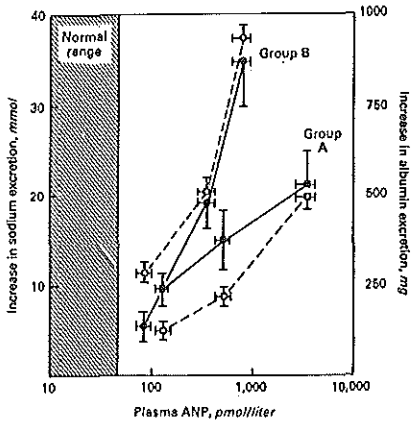
A striking observation in the present study is the effect of ANP on urinary albumin and immunoglobulin G excretion. A proteinuric effect of a high dose of ANP has been reported in normal rats [18] but to our knowledge a detailed study on the proteinuric effect of ANP in patients with nephrotic syndrome has not

Fig. 4



Relationship of the decrease in plasma volume, as calculated from changes in hematocrit, with the decrease in plasma volume, as calculated from changes in serum albumin, after 60 min of human ANP (102-126), in patients with nephrotic syndrome.

Fig.5



Relationship of the plasma level of ANP with the changes in urinary excretion of sodium (●, ■) and albumin (○, □) in patients with nephrotic syndrome following infusion of human ANP (102-106).

been published. In our study of such patients the increments in albumin excretion after ANP were proportional to the increments in fractional excretion. Increased fractional excretion of proteins may be the result of increased transglomerular diffusion, increased convection or both [19]. The diffusion component of transglomerular macromolecular transport will increase when the systemic plasma protein concentration is increased, when the ultrafiltration coefficient, K_f, is increased or when the protein concentration in the glomerular capillary bed is increased by an increased filtration fraction. Convection will change when the polarity of the basement membrane or the transcapillary pressure gradient are altered. A change in the pressure gradient will cause parallel effects on transglomerular protein filtration and glomerular filtration rate, so that fractional protein excretion is unaltered [20].

Some of these factors may be operative during infusion of ANP. First, in our patients the systemic serum concentration of albumin rose after ANP. Second, an increase in K_f has been observed in isolated perfused glomeruli of the dog when ANP was added to the perfusate [21]. ANP antagonizes angiotensin II-induced contraction of the mesangial cells [22] and this will increase the glomerular capillary surface area and thereby K_f. Finally, ANP causes an increase in filtration fraction, probably by reducing afferent arteriolar resistance and by

producing an increase in efferent arteriolar tone [23].

The effect of ANP on protein excretion resembles that of angiotensin II. Infusion of angiotensin II in the anaesthetized rat causes an increase in urinary protein excretion through an increased filtration fraction [24].

The transglomerular transport of albumin is restricted to a greater extent than would be predicted from its molecular size [20]. The glomerulus possesses a charge-selective barrier to macromolecular filtration [19], thereby impairing the transglomerular transport of albumin, which is negatively charged at physiological pH. The main barrier to filtration of negatively charged macromolecules is localized in the glomerular basement membrane at its endothelial side [20]. Although ANP receptors have been demonstrated on endothelial cells [25], there is at present no evidence that the polarity of the glomerular basement membrane can be altered by ANP. Moreover, such changes, if they occur, do not explain why the effect of ANP on albumin excretion is associated with a parallel effect on the excretion of immunoglobulin G, which is neutral at physiological pH.

At high doses, ANP caused hemoconcentration in our patients, as indicated by the increase in hematocrit and serum albumin concentration. An increase in hematocrit was already observed in the first experiments of de Bold [26], in which atrial extracts of rats were injected into intact rats. ANP may cause a shift of plasma fluid from the intravascular to the extravascular compartment [1]. In our study the reduction of plasma volume, as calculated from the changes in serum albumin concentration, was smaller than the reduction of plasma volume calculated from the changes in hematocrit (Fig 4), which suggests that the total amount of albumin within the vascular compartment is reduced by ANP. Assuming a normal plasma volume in non-edematous nephrotics, as has been reported [27], the decrease in plasma volume can be estimated from the increase in hematocrit. From this and from the change in serum albumin concentration the loss of albumin from the intravascular compartment can be calculated. For instance, after 60 min of ANP at the highest dose in groups A and B, the loss of intravascular albumin was calculated to be 3280 and 8250 g respectively, assuming an initial plasma volume of 2500 ml. This decrease in intravascular albumin was much greater than the increase in urinary excretion, which was 490 and 940 mg respectively. If we assume the maximal tubular capacity of albumin

reabsorption in 60 min to be 130 - 170 mg [6], the total amount of filtered albumin can be estimated to be 660 and 1110 mg respectively. Thus, the estimated loss of albumin from the intravascular compartment cannot be accounted for by increased filtration of this protein. It has been reported that in the nephrectomized rat the capillary absorption of extravascular fluid after intravenous administration of hyperoncotic albumin was less in animals receiving ANP than in those receiving vehicle [28]. The decreased capillary absorption of extravascular fluid in these animals could be the result of increased capillary filtration of intravascular protein. Our results suggest a similar phenomenon in nephrotic subjects.

The effect of high doses of ANP on blood pressure lasted longer (120 min) than the effect on urinary sodium and protein excretion and this long duration contrasts with the short plasma half-life of ANP (180 sec) [1]. The ANP-induced plasma volume contraction may have contributed to this long lasting effect on blood pressure. The fall in blood pressure was associated with an increase in plasma noradrenaline and heart rate, at least at the three highest doses of ANP, probably due to baroreflex-mediated increase of sympathetic tone. Plasma renin was suppressed at low doses of ANP but it was stimulated at higher doses. The suppression of renin might have been caused by a direct effect of ANP on the juxtaglomerular cells [29], whereas the rise of renin might have been caused by sympathetic stimulation in response to plasma volume contraction and the fall in blood pressure.

The lowest dose of ANP caused a rise of plasma ANP to levels slightly above the physiological range and this was already sufficient to induce natriuresis, diuresis and to increase proteinuria in our patients (Fig 5). The effective threshold dose is therefore likely to be close to the physiological secretion of ANP. As hemoconcentration occurred at the highest doses only, this effect of ANP may be pharmacological rather than physiological [30].

In conclusion, ANP appears to increase the transcapillary filtration of intravascular protein in the glomerulus and elsewhere in the body. In non-edematous patients with nephrotic syndrome the proteinuretic and natriuretic effects were already seen at low doses. Endogenous ANP may therefore have similar effects in these patients.

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Chapter 7

Effect of atrial natriuretic peptide on renal and vascular permeability in diabetes mellitus

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Abstract

Synthetic human ANP (102-126) 0.01 μg/kg/min or vehicle was intravenously infused for two hours in 10 patients with insulin dependent diabetes mellitus and micro-albuminuria (albumin excretion 20-200 μg/min) and in 10 healthy subjects. In the diabetic group, the IgG clearance was higher but both size- and charge-index as calculated from albumin and immunoglobulin clearances were equal compared to normals. The fractional clearances of small dextrans ($<$ 3.6 nm) were lower in diabetics, which was compatible with a depressed hydraulic permeability (K_f). During ANP infusion the excretion of albumin and IgG increased in the diabetic subjects (189 ± 12 to 521 ± 84 μg/min and 7.1 ± 3.5 to 21 ± 8.1 μg/min respectively; both $p < 0.05$) only. In the diabetics the clearance of dextrans $>$ 54 Å increased and our calculations indicated an increase in "Shunt-flow" (ω_0). The transcapillary escape rate of albumin, which was elevated in the diabetics at baseline, increased in the diabetic group only.
Thus ANP uncovers altered size-selectivity of the filtration barrier in a phase that is otherwise characterised by charge-selective changes only. Moreover the increased susceptibility of the glomerular capillaries in diabetics to ANP seems to be part of a more generalized capillary abnormality, because ANP also increases the transcapillary escape of albumin.

Introduction

Diabetic nephropathy is a complication affecting as many as 30% of patients with insulin dependent diabetes mellitus in the course of their disease [1]. It is characterised by proteinuria and hypertension and typically leads to loss of renal function [2]. In some patients, without clinical proteinuria as measured by reagent strip methods, a slight increase in the excretion of albumin can be detected using more sensitive assays such as RIA or ELISA [3]. This phenomenon is called microalbuminuria and is implicated as an important predictor of clinical nephropathy [4].

Several studies have examined the changes in glomerular basement membrane (GBM) permeability that underlie the increased filtration of proteins in both early and advanced diabetic renal disease [5,6,7]. Using pairs of differently charged plasma proteins, such as non-glycated versus glycated albumin and neutral IgG versus the anionic IgG_4 fraction, Deckert et al. [8] demonstrated a loss in glomerular charge selectivity early in the microalbuminuric phase of diabetic nephropathy. These changes are thought to be the result of decreased production of negatively charged constituents of the GBM such as heparan sulphate proteogycans [9]. In more advanced nephropathy, Tomlanovic et al. [6] described marked defects in the transglomerular sieving of uncharged dextrans of graded size, indicating a loss in GBM size selectivity. In a recent report, Scalding et al. [5] described similar, although less distinct, changes in size selectivity in patients with microalbuminuria. Thus the evolution of diabetic nephropathy appears to represent a continuum of progressively deranged glomerular permeability with changes in permselectivity accompanied, or preceded, by a decrease in charge selectivity of the glomerular basement membrane (GBM).

The defect in permeability to proteins is not limited to the GBM but also affects the systemic circulation since the transcapillary escape rate of both albumin and IgG is increased in microalbuminuric diabetics [10,11].

In a previous study we demonstrated that infusion of synthetic human atrial natriuretic peptide (ANP) markedly increases both proteinuria and the extravasation of plasma proteins in patients with nephrotic syndrome [12]. Thus ANP increases protein output in patients with pre-existing proteinuria. In order to investigate whether ANP can also uncover a subclinical defect in

permselectivity, we employed the infusion of ANP as a tool to probe both glomerular permeability to proteins in patients with insulin dependent diabetes mellitus and microalbuminuria. The size-selective properties of the glomerular filtration barrier were assessed by measuring fractional clearances of uncharged dextrans. Changes in charge selectivity were estimated using the fractional clearances of IgG and IgG_4. As the effect of ANP on the transcapillary passage of proteins is not limited to the glomerulus, we also measured changes in systemic capillary permeability using the transcapillary escape rate of iodinated albumin. In this fashion we attempted to detect subtle changes in permeability, not readily detectable in the basal state, by infusing ANP as a stimulus of protein filtration.

Methods

Patients

The study was performed in ten patients with insulin dependent diabetes mellitus and microalbuminuria as defined by a 24 hour urinary albumin excretion from 30 to 300 mg (9 male/1 female; age 28-54 years). We also studied ten healthy volunteers (10 male; age 21-34 years). Renal function was comparable in both groups (mean serum creatinine 80 ± 5.4 vs. 76 ± 6.9 μmol/L in diabetic and healthy subjects respectively).

No antihypertensive or non-steroidal anti-inflammatory drugs were allowed during the four weeks before the study. Each subject was studied twice, with infusion of vehicle on the first day and of ANP on the second. The experimental protocol was approved by the ethics review committee of the University Hospital Dijkzigt and written informed consent was obtained from all subjects.

Procedures

Infusion protocol:

During a four week run-in period, all medication except insulin was discontinued and 24 hour sodium excretion was measured. One week before infusion all

subjects were placed on a diet containing approximately 150 mmol of sodium. Subjects were admitted to the clinical research center at 8:00 AM after fasting for 8 hours before the study except for ad libitum water intake. Subjects did not ingest any food until the study was completed. All individuals remained supine throughout the study. They were however allowed to stand in order to pass urine. At the start of the infusion (-150 min) all subjects received an oral water load of 20 ml/kg body weight. In order to achieve a steady state urine output, the urinary volume was replaced by drinking tap water. Plastic cannulas were inserted into an antecubital vein of each arm. Insulin, dextrans and radiolabeled clearance markers were infused in one arm, while blood samples were drawn from the other arm.

After a 90 min. equilibration the bladder was emptied by voiding (-60 min). Urine was collected during two carefully timed 30 minute periods (-60 to -30 min. and -30 to 0 min.) that served as baseline periods. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured in each of the clearance periods. During the second baseline period a mid-point plasma sample was taken to allow the calculation of fractional clearances. Following the baseline periods, human ANP (102-126; Wy 47.663, Wyeth Research Laboratories, Philadelphia, Pennsylvania, USA) in vehicle (NaCl 0.9% containing 5% human serum albumin) or placebo (vehicle) was infused intravenously for 120 minutes at a dose of 0.01 μg/kg/min. Urine was subsequently collected during four 30 minute intervals. During the last interval (90 to 120 min.) a second dextran clearance was performed as described above. At 60 minutes after the start of the infusion of ANP or vehicle, a single shot of ^{125}I-albumin was given.

In the diabetic subjects the usual morning dose of insulin was omitted and a constant infusion of insulin (1% of the daily dose per hour) in glucose 5% (50 mg/kg/min) was given from the start of the study. Subsequently, blood glucose concentrations were measured at 30 minute intervals and kept between 4 and 8 mmol/L by small intravenous injections of insulin or glucose 50%.

Clearance studies:

In order to determine GFR and ERPF, we measured the renal clearances of inulin (Inutest, Laevosan-Gesellschaft, Linz/Donau, Austria) and ^{131}I-orthoiodohippurate (Amersham, UK) respectively. The standard formula was used

to calculate the clearance values. In order to achieve stable plasma concentrations of the clearance markers, the priming dose and sustaining infusion rate were adjusted to renal function as previously described [13]. This adjustment was based on prior estimation of renal function using the equation of Cockcroft and Gault [14]. Fractional clearance (F_{CL}) of macromolecules was calculated using the equation:

$$F_{CL} = [(U/P) / (U\text{-inulin}/P\text{-inulin})]$$

in which U and P are the urinary and plasma concentrations of the macromolecule. In each urine and plasma sample we also measured albumin, IgG and IgG_4 concentrations. The selectivity index (SI) was calculated as clearance of IgG divided by that of albumin. In general, proteinuria is considered to be selective when SI is below 0.2 [15]. IgG and IgG_4 are of the same size but are differently charged at physiological pH. Therefore, the clearance of IgG divided by that of IgG_4 can be used as a measure of charge selectivity (charge index; CI) [8]. The interpretation of protein clearance measurements is complicated by tubular reabsorption of the proteins.

Dextran clearances were performed as follows. At time -90 min. a 200 mg dose of dextran-10 (Promiten®, NPBI, Emmercompascuum, The Netherlands) was given in order to prevent anaphylactic reactions to the subsequent doses. After ten minutes, 50 mg/kg dextran-70 (Macrodex®) was given in a 10 minute infusion, followed by a constant infusion of dextran-40 (Rheomacrodex®) at 100 mg/min, which was continued throughout the whole observation period.

Plasma volume and the transcapillary escape rate of albumin were calculated from ^{125}I counts in plasma samples taken 10, 15, 20, 30, 45 and 60 minutes after the injection of ^{125}I-albumin, as described by Parving et al. [13]. To correct for the higher baseline levels, a larger dose (296 vs 148 kBq) of ^{125}I-albumin was used on the second day of study.

Analytical methods

Blood pressure was determined at 5 minute intervals, with an oscillometric device (Accutorr, Datascope Corp, Paramus, New Jersey, USA). The mean of the last three readings in each study period was used for analysis. Inulin concentrations were measured using the resorcinol assay [16]. Enzyme linked immunosorbent

assays (ELISA) were developed for the determination of albumin, retinol binding protein (RBP), IgG and IgG_4 in plasma and urine. In brief, Maxisorb test plates (Nunc, Roshilde, Denmark) were coated with a monoclonal antibody directed against either albumin (A001), RBP (A040), IgG (MH16-01-M06) or IgG_4 (MH164-1-M05; Central Laboratory of Blood Transfusion, Amsterdam, The Netherlands). After overnight incubation with sufficiently diluted samples the plates were thoroughly washed. A second, alkaline phosphatase labelled, monoclonal antibody against either albumin (P356), RBP (P304) or IgG (P214; Dakopatts, Glostrup, Denmark) was then added. After washing, the plates were incubated with Enzymun-test (Boehringer Mannheim, Germany) as substrate. Reference serum supplied by the Central Laboratory of Blood Transfusion was used as the standard. The sensitivity of the assays was 1 ng/ml. Interassay coefficients of variation were 5 to 7 % in ten subsequent assays. Dextran was assayed in protein-free filtrates of plasma and urine (trichloric acetic acid 40%). These filtrates were separated into narrow fractions by gel-permeation chromatography using the method described by Granath et al. [17]. A sephacryl S-300 column (Pharmacia, Upsala, Sweden) of 180 ml bed-volume and 90 cm in length was used. The eluent was a 0.01 M Tris buffer at pH 7.0 with 0.15 M NaCl containing 1 mM EDTA. Blue dextran (Mw 2,000,000 D) was used to determine void volume (V_o), and the column was calibrated using dextran T10, T40 and T70 (Pharmacia). The fractional volume available to the solute (K_{AV}) was then calculated from:

$$K_{AV} = (V_e - V_o)/(V_t - V_o)$$

where V_e is the volume necessary to displace a solute from the column, and V_t is the total bed-volume of the gel column. Effective molecular radii for the individual dextran fractions were calculated from K_{AV}. After gel permeation chromatography, eluted fractions were assayed for dextran using the anthrone method of Scott and Melvin [18].

The colloid osmotic pressure in the afferent arteriole (π_a) was assumed to be equal to systemic colloid osmotic pressure, and was calculated from plasma protein concentrations using the formula of Landis and Pappenheimer [19].

A direct radioimmunoassay was used to measure naturally occurring active renin and serine protease-activated prorenin as described previously [20].

Analysis of membrane pore structure

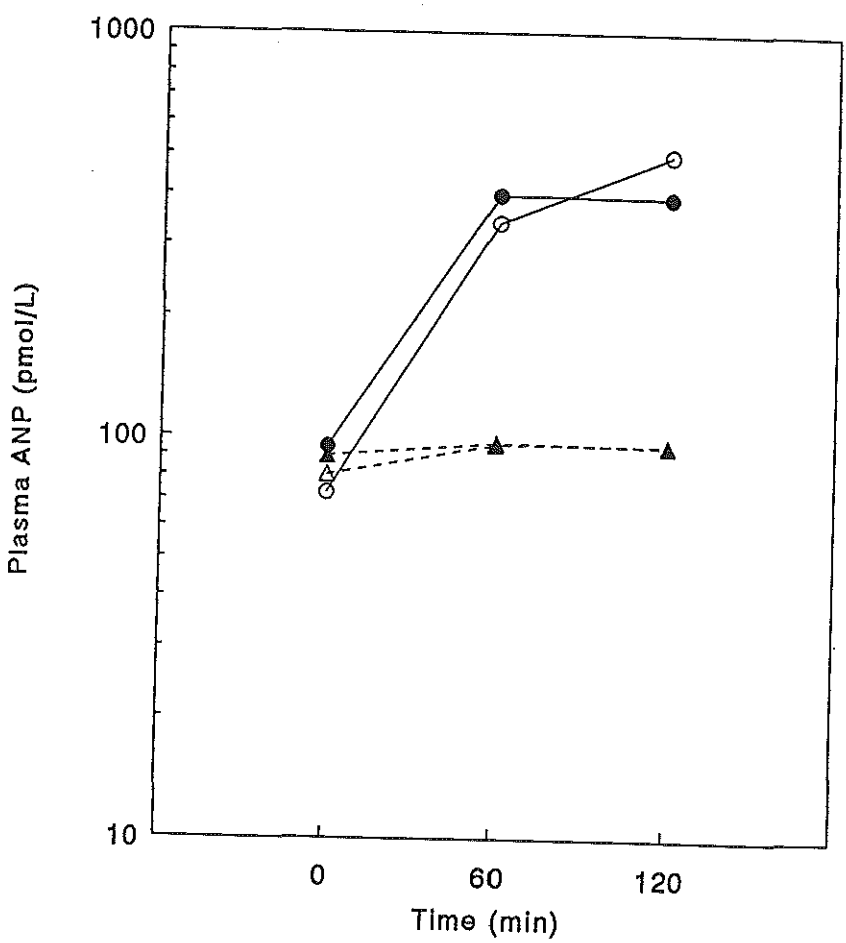
To analyze the size-selective properties of the glomerular barrier, we used a heteroporous model of the glomerular capillary wall, as described by Deen et al [21]. This model has been shown to provide the most satisfactory representation of dextran sieving. In this model the major portion of the capillary wall is perforated by restrictive cylindrical pores of identical radius (r_0). In addition, this model assumes a parallel 'shunt pathway' that does not discriminate on the basis of dextran size, and through which a small fraction (ω_0) of the filtrate volume passes. The size of this fraction is not merely dependent on the properties of the capillary wall, but also on intracapillary oncotic pressure. Therefore a quantity is introduced that is closely related to ω, but that is independent of oncotic pressure and therefore characteristic of the membrane per se. This quantity (ω_0) is the fraction of the volume flux that would pass through the shunts if plasma proteins were absent. The membrane barrier to dextrans is fully characterised by r_0, ω_0 and K_f, where K_f is the product of effective hydraulic permeability and glomerular capillary surface area. These variables can be estimated using curve fitting techniques. In order to make these calculations an estimate of the transmembrane hydraulic pressure difference (ΔP) is required. Since ΔP cannot be measured directly in humans, a value must be assumed in order to calculate the basement membrane parameters. In normal humans ΔP is predicted to be close to 35 mmHg [22]. We therefore calculated intrinsic basement membrane parameters using a ΔP of 35 mmHg. Since experimental studies have indicated that ANP may increase ΔP [23], we calculated basement membrane parameters after ANP using a ΔP of both 35 and 40 mmHg. Although we have no direct measurements of ΔP in humans, this does not seriously hamper the use of dextran sieving as a measure of permselectivity because the calculation of basement membrane characteristics is relatively insensitive to changes in filtration pressure [11]. Thus, although the quantitative changes in basement membrane parameters may not be exact, this method does give at least semi-quantitative information on the direction of such changes.

Statistical analysis

Baseline data in diabetics and healthy subjects were compared using a one-way

ANOVA. When this yielded a significant F value, data were analyzed by the Student Neumann Keuls (SNK) test. Response to treatment (Tables 1 and 2) was evaluated using a two-way ANOVA with repeated measures comparing treatment effect to vehicle. Differences in treatment effect between diabetics and healthy subjects were estimated by means of the Student's t test, comparing the total area under the curve (ANP minus vehicle). Dextran data and basement membrane characteristics were compared using one-way ANOVA and SNK tests. Urinary excretion data were not normally distributed and were therefore log-transformed before analysis. All calculations were performed using the SPSSPC+ statistical software package. Data are expressed as means ± SEM.

Fig. 1



Results

The infusions were carried out without complications and all subjects completed the protocol. No side-effects were observed during either infusion. In the diabetic patients metabolic control was moderate, indicated by an average HbA_1C of 9.6 $\pm$2.1%.

Baseline ANP concentrations were not different in diabetic and healthy subjects (80.9$\pm$14.8 vs. 90.0$\pm$15.1 pmol/L respectively). During infusion of human ANP (102-126), ANP levels rose in both groups (Figure 1). No difference was observed between the maximal ANP concentrations that were reached in each group.

Effects of ANP on renal hemodynamics, and urinary sodium and protein excretion.

Blood pressure was significantly higher in the diabetic group than in the healthy subjects (systolic 139.1$\pm$6.6 vs. 124.4$\pm$3.5 mmHg; $p<0.05$ and diastolic 78.3 $\pm$4.5 vs 70.5 $\pm$2.5 mmHg; $p<0.05$). No significant change in systolic or diastolic blood pressure occurred during the infusion of ANP in either group.

Urinary volume (V) at baseline, before the start of the infusion, was lower in the diabetic subjects (Table 1). During ANP, V increased significantly in both groups, but no differences in the effect of ANP were observed.

Both excretion ($U_{Na}V$) and the fractional excretion of sodium (FE_{Na}) at baseline tended to be higher in the diabetic subjects, but neither difference reached statistical significance. Following ANP, $U_{Na}V$ and FE_{Na} increased significantly in both groups. There was no difference in effect between the two groups.

At baseline both groups were comparable with regard to glomerular filtration rate, effective renal plasma flow and filtration fraction (Table 2). No significant changes in renal hemodynamics were observed after ANP, in either diabetic or healthy subjects.

At baseline, no significant differences in either SI or CI were observed between diabetics and healthy subjects (0.20 $\pm$ 0.05 vs. 0.16 $\pm$ 0.04 and 2.4 $\pm$ 0.6 vs. 3.3 $\pm$ 0.4 respectively). During infusion of ANP the excretion of albumin and IgG rose in the diabetic subjects only (Figure 2). The percentual rise in albumin excretion during ANP was proportionate to baseline albuminuria. Fractional

Table 1

			BASELINE	ANP 0.01 μg/kg/min or VEHICLE			
				30 min.	60 min.	90 min.	120 min.
V (ml/min)	NORMALS	*vehicle*	14.9 ± 1.2	16.6 ± 1.0	14.7 ± 1.2	14.8 ± 0.9	15.6 ± 1.1
		ANP	15.0 ± 1.0	16.4 ± 1.0	16.5 ± 1.1	21.0 ± 2.1	14.5 ± 1.1 [b]
	DIABETICS	*vehicle*	10.8 ± 1.5 [a]	12.5 ± 1.4	11.1 ± 0.91	11.4 ± 1.0	11.6 ± 1.6
		ANP	8.9 ± 0.81 [a]	11.5 ± 1.2	12.5 ± 1.3	13.1 ± 1.3	14.6 ± 1.8 [b]
$U_{Na}V$ (μmol/min)	NORMALS	*vehicle*	153.4 ± 18.8	172.7 ± 13.7	181.5 ± 12.7	184.2 ± 14.5	191.8 ± 20.9
		ANP	138.2 ± 19.3	177.2 ± 26.7	241.3 ± 49.6	333.3 ± 56.5	238.4 ± 18.7 [b]
	DIABETICS	*vehicle*	256.2 ± 60.7	223.5 ± 68.8	195.8 ± 55.5	211.3 ± 61.4	243.7 ± 96.0
		ANP	190.0 ± 80.9	221.5 ± 74.2	292.7 ± 87.0	328.2 ± 83.6	393.9 ± 122.5 [b]
FE_{Na}	NORMALS	*vehicle*	0.96 ± 0.11	1.13 ± 0.15	1.31 ± 0.13	1.19 ± 0.13	1.14 ± 0.12
		ANP	0.96 ± 0.15	1.20 ± 0.23	1.76 ± 0.40	1.79 ± 0.37	1.61 ± 0.14 [b]
	DIABETICS	*vehicle*	1.43 ± 0.40	1.55 ± 0.49	1.46 ± 0.45	1.71 ± 0.57	1.68 ± 0.65
		ANP	1.28 ± 0.52	1.69 ± 0.65	2.06 ± 0.71	2.45 ± 0.83	2.56 ± 0.86 [b]

[a] $p<0.05$ vs normals, [b] $p<0.05$ ANP vs vehicle

clearances of albumin and IgG in the diabetics increased by 126 and 81% respectively and the fractional clearance of IgG_4 rose by 75%. Neither size nor charge selectivity indices were significantly altered by ANP.
Before the start of the ANP infusion, the excretion rates of RBP were not different in normals and diabetics (69 $\pm$19.4 and 85 $\pm$27.3 ng/ml respectively). Infusion of ANP did not affect the excretion rates of RBP in either group.

Effects of ANP on dextran clearances and glomerular permeability characteristics.
Fractional clearances of polydisperse dextrans in microalbuminuric diabetics differed markedly from normals. Dextrans with a radius smaller than 3.6 nm showed significantly lower fractional clearance in the diabetics (Figure 3). No significant difference was observed in fractional clearance of larger dextrans. These findings at baseline were compatible with a depressed value of K_f in the diabetic subjects. The shunt-flow parameter ω_0 tended to be higher in diabetics, although this failed to reach statistical significance.
ANP did not alter the fractional clearance of smaller dextrans. In contrast to healthy individuals, in which no changes in dextran sieving occurred after ANP, in diabetics the fractional clearance of dextrans with a stokes radius larger than 5.2 nm was significantly increased (Figure 3). After ANP, calculated basement membrane parameters in the diabetic subjects indicated a significant increase in the "shunt-flow" parameter ω_0 with no significant alteration of either r_0 or K_f (Table 3). Like the change in albuminuria, the ANP-induced change in ω_0 was proportionate to baseline albuminuria (Figure 4).

Effects of ANP on plasma volume and the transcapillary escape rate of albumin.
Plasma volume (PV) was not significantly different in diabetic and healthy subjects (3128 $\pm$588 and 3273 $\pm$371 ml/1.73 m^2 respectively), and did not significantly alter after ANP. Transcapillary escape rate of albumin (TER_{alb}) was significantly higher in the diabetic patients (9.2 $\pm$1.4 vs 5.4 $\pm$2.4 %/hour; $p<0.05$). During infusion of ANP no change in TER_{alb} occurred in normals. In the diabetics however, TER_{alb} rose to 13.4 $\pm$2.6 %/hour ($p<0.05$, Figure 5). Hematocrit also rose in the diabetic group only (0.38 $\pm$0.03 to 0.41 $\pm$0.02; $p<0.05$).

Fig. 2

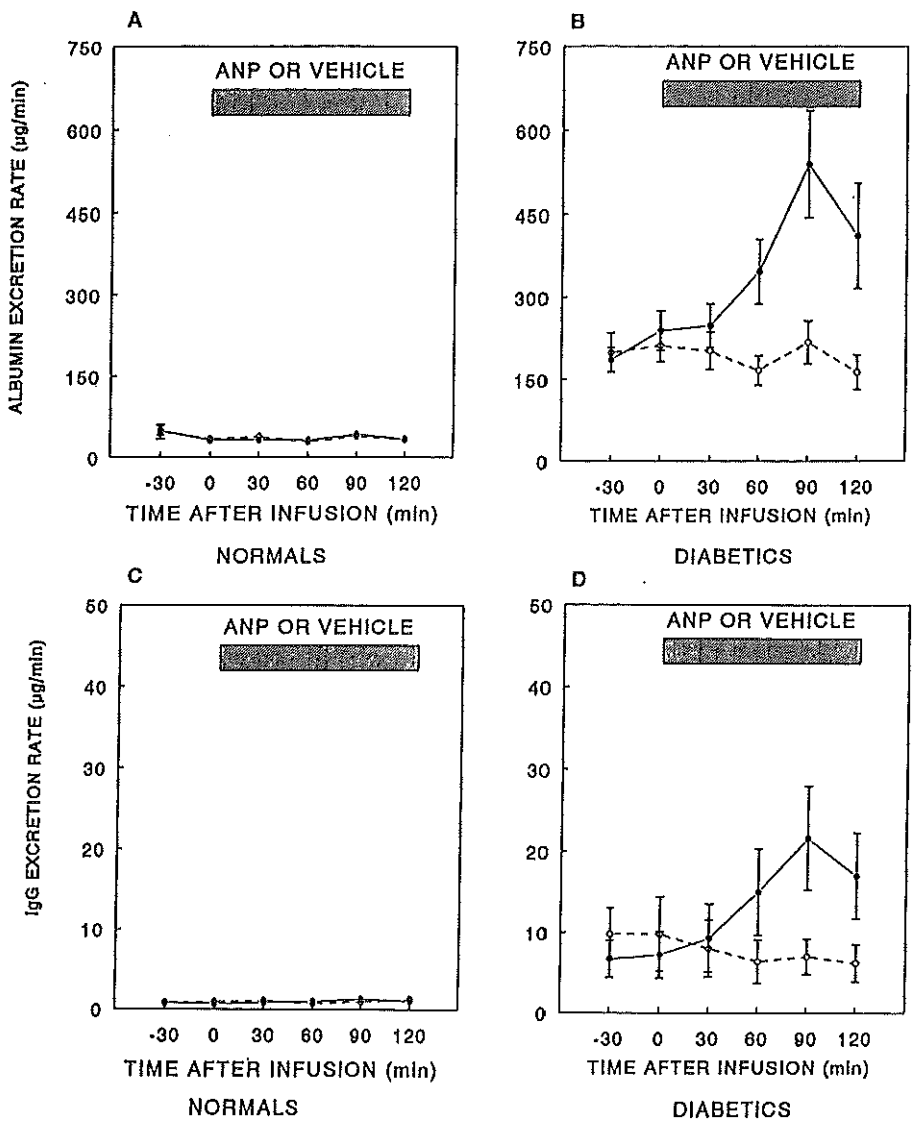

A
B
C
D
ANP OR VEHICLE
ALBUMIN EXCRETION RATE (µg/min)
IgG EXCRETION RATE (µg/min)
750
600
450
300
150
0
50
40
30
20
10
-30 0 30 60 90 120
TIME AFTER INFUSION (min)
NORMALS
DIABETICS

Table 2

			BASELINE	ANP 0.01 μg/kg/min or VEHICLE			
				30 min.	60 min.	90 min.	120 min.
GFR (ml/min)	NORMALS	*vehicle*	114 ± 7.1	118 ± 6.9	104 ± 6.1	113 ± 6.3	123 ± 5.9
		ANP	108 ± 5.5	113 ± 5.6	101 ± 3.5	114 ± 7.2	110 ± 5.0
	DIABETICS	*vehicle*	114 ± 9.9	116 ± 10.1	106 ± 6.5	112 ± 13.0	112 ± 10.9
		ANP	116 ± 7.7	115 ± 11.9	114 ± 10.0	122 ± 14.0	120 ± 9.7
ERPF (ml/min)	NORMALS	*vehicle*	585 ± 41.3	582 ± 35.5	540 ± 45.9	563 ± 31.7	643 ± 27.5
		ANP	527 ± 34.5	575 ± 28.4	535 ± 31.1	579 ± 43.5	579 ± 43.7
	DIABETICS	*vehicle*	603 ± 52.5	590 ± 50.2	535 ± 35.1	563 ± 54	553 ± 52.5
		ANP	617 ± 37.9	616 ± 49.4	552 ± 38.0	544 ± 53.3	619 ± 61.2
FF	NORMALS	*vehicle*	0.20 ± 0.01	0.21 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.19 ± 0.01
		ANP	0.21 ± 0.01	0.20 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.20 ± 0.01
	DIABETICS	*vehicle*	0.21 ± 0.02	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.21 ± 0.01
		ANP	0.21 ± 0.02	0.19 ± 0.01	0.21 ± 0.01	0.23 ± 0.01	0.20 ± 0.01

[a] $p<0.05$ vs normals, [b] $p<0.05$ ANP vs vehicle

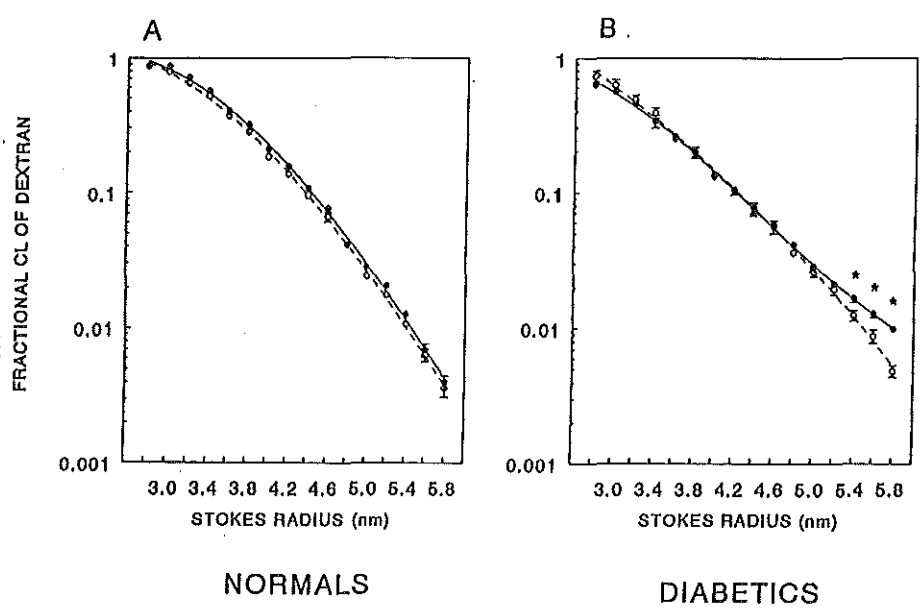
The plasma concentrations of active renin were comparable in both groups. Plasma prorenin was significantly higher in the patients with diabetes mellitus (522 ±511 vs 122 ±47 pg/ml; $p<0.05$). Plasma renin and prorenin did not change after ANP.

Discussion

The present study explores the effects of ANP on glomarular barrier function in microalbuminuric diabetic subjects.

At baseline microalbuminuric diabetics had higher IgG excretion rates than healthy subjects. The finding of increased excretion of IgG indicates that loss of size-selectivity could contribute to proteinuria in patients with microalbuminuria. The selectivity index however was the same in both groups. Because this index

Fig. 3



is based on the measurement of the fractional clearances of plasma proteins and because these clearances are influenced by tubular protein reabsorption, we also studied size-selectivity by measuring the clearances of dextran molecules of broad size distribution. At baseline, dextran sieving in micro-albuminuric diabetics differed significantly from that in healthy subjects. This difference was confined to the dextrans with a low molecular weight. These findings are similar to those reported by Scalding et al. [11].

By applying a mathematical model to the dextran clearance data, we could explain the difference in fractional clearance of small dextrans by a reduction in the ultrafiltration coefficient K_f. Such reductions in K_f might result from decreased hydraulic permeability of the filtration barrier, or from a decrease in filtration surface area. An increase in GBM thickness has been described early in the course of diabetic renal disease [24]. A decrease in the total GBM surface area seems a less likely explanation for the reduction in K_f because early diabetic nephropathy is characterised by renal hypertrophy [25]. At baseline, GFR was not increased in the diabetic subjects. This could suggest that the patients studied had already passed through the phase of hyperfiltration, possibly secundary to the reduction in K_f.

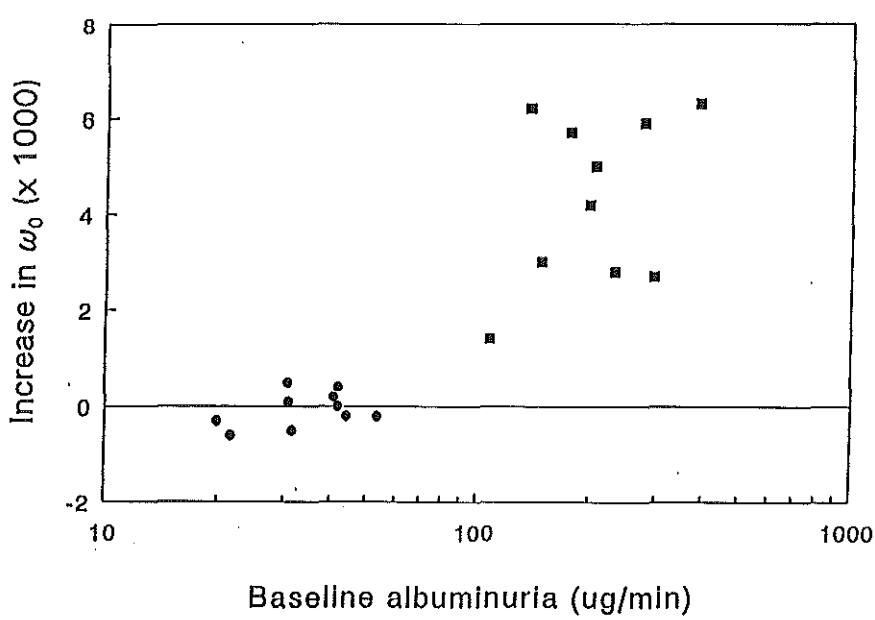
Table 3

		ΔP	K_f	r_o	ω_o
		mmHg	ml.min^{-1}.mmHg	Å	x 10^{-3}
NORMALS	*vehicle*	35	13.3 ± 0.9	56.9 ± 0.01	1.4 ± 0.1
	ANP	35	13.6 ± 0.8	56.8 ± 0.01	1.3 ± 0.1
		40	12.4 ± 0.7	56.8 ± 0.01	1.5 ± 0.2
DIABETICS	*vehicle*	35	9.8 ± 1.3 [a]	55.7 ± 0.01	1.8 ± 0.3
	ANP	35	10.1 ± 1.2	55.9 ± 0.01	6.4 ± 0.4 [ab]
		40	9.1 ± 1.1 [a]	55.9 ± 0.01	6.1 ± 0.4 [ab]

[a] $p<0.05$ vs normals, [b] $p<0.05$ vs vehicle

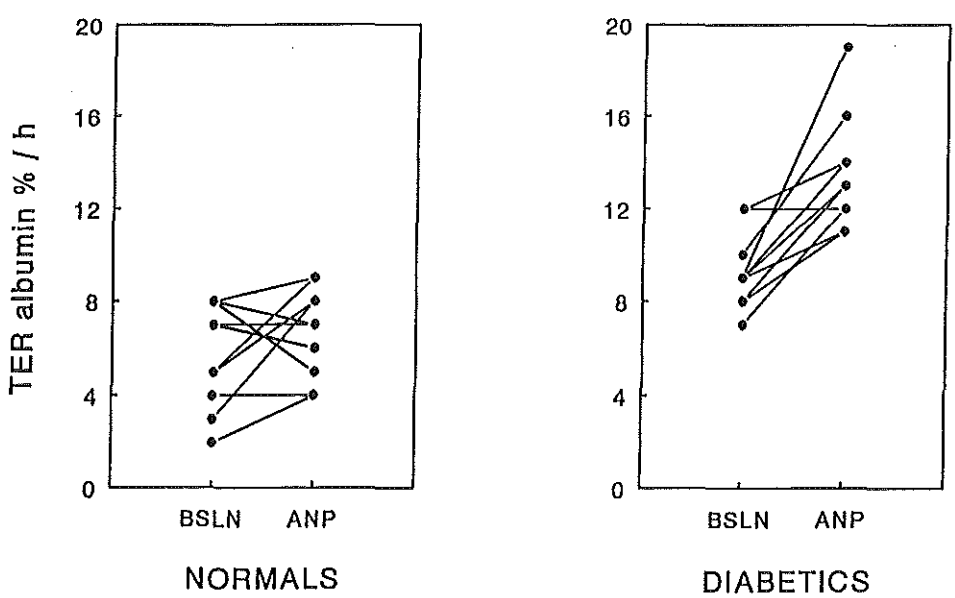
At baseline, the shunt-flow parameter ω_0 in diabetics did not differ from that in normals. Thus, in order to explain the increased protein clearances, one has to assume a decrease in charge selectivity of the glomerular barrier. Reduced charge selectivity has been observed in diabetic subjects with albumin excretion rates as low as 30 to 50 mg/24 hrs. In our study charge index, calculated as the quotient of fractional IgG and IgG_4 clearances, tended to be lower in the diabetics. This tendency did not reach statistical significance. A slight alteration in charge selectivity however, not readily detectable in our small group of patients, could have a substantial effect on albumin clearance. Also, our results may have been influenced by changes in tubular protein reabsorption, which is also charge dependent. However, the excretion rate in retinol binding protein, a low-molecular-weight protein measured to asses differences in tubular reabsorbtion, was not different in the diabetic subjects.

Fig. 4



At baseline our diabetic subjects had a significantly higher transcapillary escape rate of albumin (TER_{alb}) as compared to normals. A similar increase has been demonstrated in previous studies by Parving et al. [11]. Tucker et al. [26] described an increase in TER_{alb} in rats with streptozotocin-induced diabetes mellitus, which occurred within 24 hours, well before the onset of albuminuria. In this study baseline ANP levels were normal in patients with micro-albuminuric insulin dependent diabetes mellitus. Other studies reported elevated [27][28] or normal [29][30] plasma ANP levels in such patients. Elevated ANP was mainly observed in patients with autonomic insufficiency [30], or with poor metabolic regulation [30] or advanced nephropathy [31]. In the early micro-albuminuric phase of diabetic renal disease ANP levels were generally normal [31].
Our infusion of ANP increased circulating ANP levels approximately sixfold. The plasma levels during infusion were comparable to those observed in nephrotic subjects at the same dose of ANP [12], and were well within the range observed

Fig. 5



in pathological states such as congestive heart failure [32]. The kinetics of ANP appeared to be comparable in diabetics and healthy volunteers.

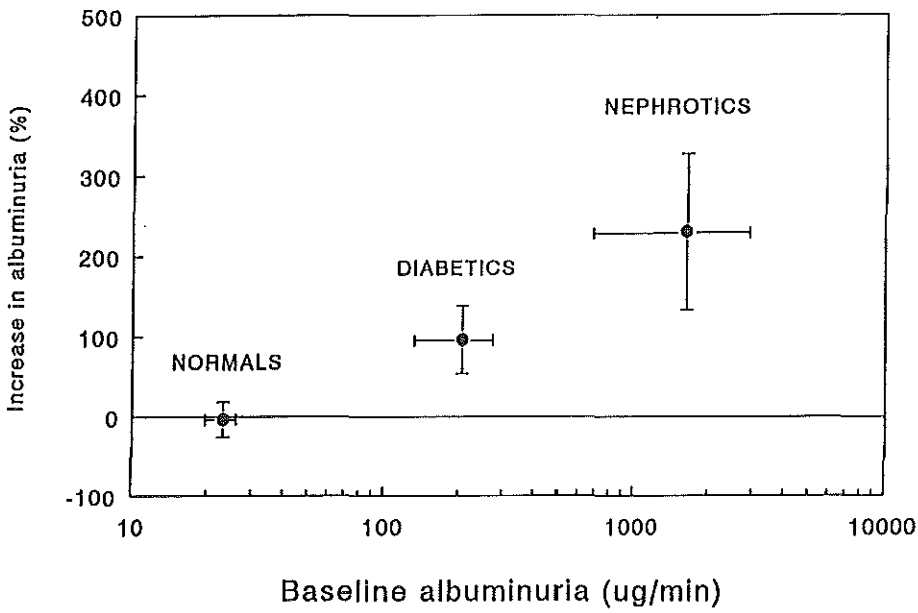
The ANP dose we used in the present study was chosen because it was the lowest dose inducing a consistent rise in proteinuria in patients with nephrotic syndrome [12]. Furthermore, no changes in protein excretion could be induced in healthy subjects with this dose of ANP [33], although a proteinuric effect of ANP has been described in normals at higher doses [34]. With the present dose therefore, we hoped to uncover subtle alterations in glomerular barrier function of the diabetics.

Indeed, the most striking finding of this study was the effect of ANP on albumin and IgG excretion in patients with diabetes. In micro-albuminuric diabetics ANP caused a three-fold rise in the excretion of albumin and IgG whereas albumin and IgG excretion did not change in healthy subjects. The increase in protein excretion observed in the diabetic subjects was not associated with changes in the calculated values of size selectivity or charge-index. In contrast, the fractional clearances of uncharged dextrans were significantly altered in the diabetics. Following the administration of ANP, clearance of large dextrans ($>$ 5.2 nm) increased, and our calculations of K_f and ω_0 indicated that the increased clearance of large dextrans was caused by increased ω_0. This parameter represents the percentage of flow through "shunt-like" pores that are not size restrictive [21]. Thus ANP reduced the size-selective properties of the glomerular filtration barrier in diabetics. The calculated increase in ω_0 was large enough to fully explain the increases in both F_{CL} albumin and F_{CL} IgG we observed in these patients. No changes in dextran sieving were observed in healthy subjects during infusion of ANP.

Therefore, the ability of ANP, at the dose we used, to increase flow through the non-restrictive "shunt-like" pores appears to be related to baseline albumin excretion rates. Indeed, when the effects of ANP on albumin excretion in patients with nephrotic syndrome were also considered, the relationship between baseline albuminuria and the albuminuric response to ANP could be extended (Figure 6).

In the present study ANP increased TER_{alb} in diabetic subjects. An increase in hematocrit following ANP administration has been well documented [11][35] and also occurs in nephrectomized animals [36]. Williamson et al. [37] studied the mechanism of ANP induced hemoconcentration in rats. Increased permeability

Fig. 6



to ^{125}I-bovine serum albumin was observed in most tissues and was explained by increased capillary pressure. It is possible that the effect of increased net transmembrane pressure is amplified by a simultaneous increase in hydraulic permeability as has been observed in the frog [38]. Since an increase in TER_{alb} in response to ANP was only observed in diabetics and not in healthy subjects, it seems that the changes in permeability of the glomerular capillaries in response to ANP are part of a more general microvascular abnormality.

The dose of ANP we used had no effect on blood pressure. Blood pressure changes are therefore unlikely to be responsible for the observed proteinuric effects of ANP.

During ANP infusion urine output and natriuresis increased to an equal extent in both groups. This is contrary to most studies, in which a blunted effect of ANP on water and sodium excretion was seen in diabetics subjects [29]. The tendency,

although not significant, towards a higher average salt intake in our group of diabetics (24 hour sodium output 200± 69 mmol/day vs. 142 ± 51 mmol/day in the healthy subjects) might partly explain this discrepancy, because salt loading is known to increase the renal sensitivity to ANP [39]. We observed no changes in renal hemodynamics in response to ANP, neither in diabetics nor in normals. Slight changes in FF, although not readily detectable in small groups of subjects, can have significant effects on the renal handling of proteins [40]. An increase in FF has been observed in other studies, in which doses of ANP were administered comparable to the dose we used [41].

The effect of ANP on proteinuria appears to be related to the preexisting level of proteinuria. Possibly an ANP induced increase in filtration pressure increases the flow through non-selective pores or distorts the GBM structure. Increased hydraulic pressure in the glomerular capillaries has been observed in animal studies [23] and is supposed to be a result of afferent vasodilation and efferent vasoconstriction in the glomerular capillaries [42]. Therefore, the response to ANP infusion in diabetics may point to a derangement of GBM structure that is not clinically evident under normal circumstances. Indeed ultrastructural studies have shown decreased cross-linking of GBM collagen fibres [43] which might be related to a decreased sulphated glyosaminoglycan content [44]. It is commonly held that the sulphated glyosaminoglycans are the most important component of the negative charge barrier, which is assumed to be instrumental in the pathogenesis of diabetic microalbuminuria [45]. Alternatively, ANP could affect the permeability of the filtration-slit membranes which are attached to the podocytes, which possess abundant numbers of ANP-receptors [46]. In summary, ANP uncovers altered size-selectivity of the filtration barrier in a phase that is otherwise characterised by charge-selective changes only. Moreover the increased susceptibility of the glomerular capillaries in diabetics to ANP seems to be part of a more generalized capillary abnormality, because ANP also increases the transcapillary escape of albumin.

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ACE-inhibition does not correct the subclinical defect in renal and vascular permeability made apparent by ANP infusion in diabetes mellitus

R Zietse, FHM Derkx, W Weimar and MADH Schalekamp.
Submitted.

Abstract

In diabetes mellitus a selective increase in the excretion of albumin generally precedes the occurrence of demonstrable loss of glomerular size selectivity. However, even in this (microalbuminuric) phase of diabetic nephropathy a defect in glomerular barrier function can be demonstrated during infusion of atrial natriuretic peptide (ANP). The aim of this study was to investigate whether angiotensin converting enzyme (ACE) inhibition could prevent the proteinuric response to ANP in these patients. Therefore we performed infusions of ANP (0.01 μg/kg/min) in ten patients with insulin-dependent diabetes mellitus and microalbuminuria (urinary albumin excretion 90$\pm$44 mg / day), both before and after one month of treatment with enalapril (20 mg once daily). Despite a 40 % reduction in proteinuria, ACE-inhibition did not prevent the ANP-induced increase in protein excretion. Both before and during ACE inhibition ANP infusion resulted in a significant increase in the fractional excretion of large dextran molecules, which is compatible with an increase in flow through large unrestrictive "shunt" pores. ANP infusion also induced an increase in the transcapillary escape rate of albumin and ACE-inhibition also failed to prevent this effect of ANP on peripheral capillary permeability.
We conclude that ACE inhibition during one month does not correct the capillary

barrier function defect in patients with diabetes mellitus and microalbuminuria that is unmasked by ANP infusion.

Introduction

Early in the course of diabetic nephropathy only small amounts of albumin are excreted in the urine which can be detected with sensitive assays [1]. This state is referred to as microalbuminuria and its presence is indicative of an increased risk of overt nephropathy [2]. The proteinuria in microalbuminuric diabetes is thought to result from a derangement in the charge-selective properties of the glomerular filtration barrier [3]. A reduced content of negative charge constituents, such as heparan sulphate, of the glomerular basement membrane has been demonstrated in diabetic rats [4], as well as in patients with diabetic nephropathy [5]. Despite the fact that this early phase of diabetic nephropathy is often referred to as microalbuminuria, the protein excreted by these patients does not consist exclusively of albumin [6]. Even at this stage, the excretion of larger proteins such as IgG is also increased. Thus alteration in glomerular barrier function cannot be explained by an alteration in charge selectivity only, but an early dysfunction of glomerular size-selectivity must also be assumed.
Progression to macroproteinuria is accompanied by a progressive derangement of glomerular size-selectivity, as has been demonstrated by clearance studies using uncharged polydisperse dextran molecules [7].
Recently we demonstrated that infusion with atrial natriuretic peptide (ANP) is able to induce a loss of size-selectivity in microalbuminuric diabetes, in a phase that is otherwise characterized by charge-selective changes [8]. Thus it uncovers a subclinical defect in glomerular barrier function.
Treatment with angiotensin converting enzyme (ACE) inhibitors reduces protein excretion in subjects with diabetes [9][10], as well as in proteinuria due to other forms of renal disease [11]. At least part of this effect may be related to systemic blood pressure lowering [12]. A specific effect on the kidney could also be involved. This might result from either a reduction in ΔP [13] or might be due to remodelling of the filtration barrier [14]. Morelli et al. [10] reported that 3

months of treatment with an ACE inhibitor induced a shift of all pores towards a smaller size resulting in a generalized enhancement of barrier size-selectivity. These findings cannot be explained by a selective reduction of filtration pressure, because this is predicted to result in an increased clearance dextrans of relatively small size only.

The present study was undertaken to investigate whether ACE-inhibition could correct the defect in glomerular barrier function that is made apparent by infusion of ANP. If so, this would lend strength to the hypothesis that ACE-inhibition alters the glomerular filtration barrier structurally rather than through changes in renal hemodynamics.

Methods

Patients

The study was performed in ten patients with insulin-dependent diabetes mellitus and microalbuminuria as defined by a 24 hour urinary albumin excretion from 30 to 300 mg (Table 1). None of the subjects studied were treated with

Table 1

Clinical characteristics

	n = 10
Male/Female	9/1
Age (years)	51.9 ± 7.5
Weight (kg)	74.3 ± 2.4
Duration of diabetes (months)	97.3 ± 11.2
Glycated Hb (%)	9.6 ± 2.1
Serum creatinine (μmol/L)	76.1 ± 6.9
Urinary albumin excretion (mg/day)	90.1 ± 43.5
Urinary sodium excretion (mmol/day)	200 ± 69.3

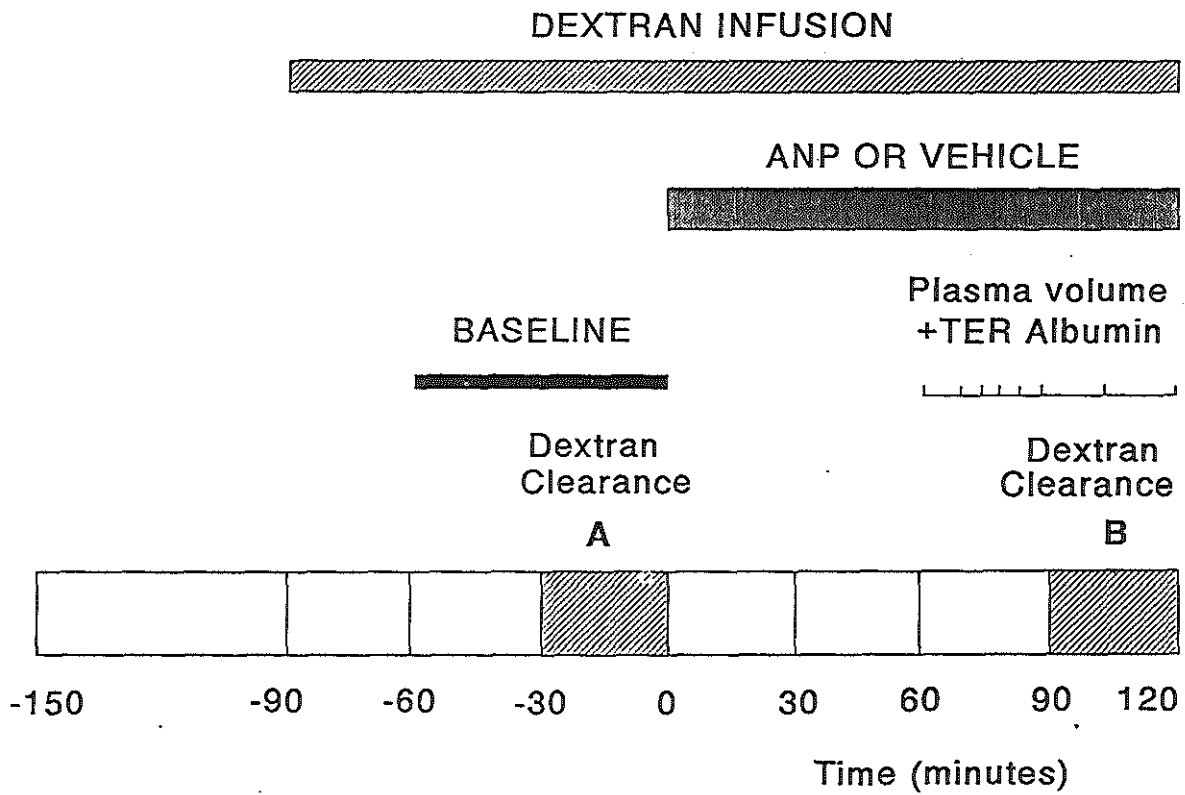

DEXTRAN INFUSION
ANP OR VEHICLE
Plasma volume
+TER Albumin
BASELINE
Dextran
Clearance
A
Dextran
Clearance
B
-150
-90
-60
-30
0
30
60
90
120
Time (minutes)

Fig 1.

antihypertensive drugs prior to inclusion into the study. No non-steroidal anti-inflammatory drugs were allowed during the four weeks before the study.
Each subject was studied four times. At baseline the subjects received an infusion of ANP and one of vehicle on two consecutive days. After four weeks of treatment with 20 mg/day enalapril the ANP and vehicle infusions were repeated. All infusions were carried out in random order. The characteristics of glomerular permeability were compared to ten healthy control subjects, comparable except for the presence of diabetes, which were described in a previous report [8]. The experimental protocol was approved by the ethics review committee of the University Hospital Dijkzigt, and written informed consent was obtained from all subjects.

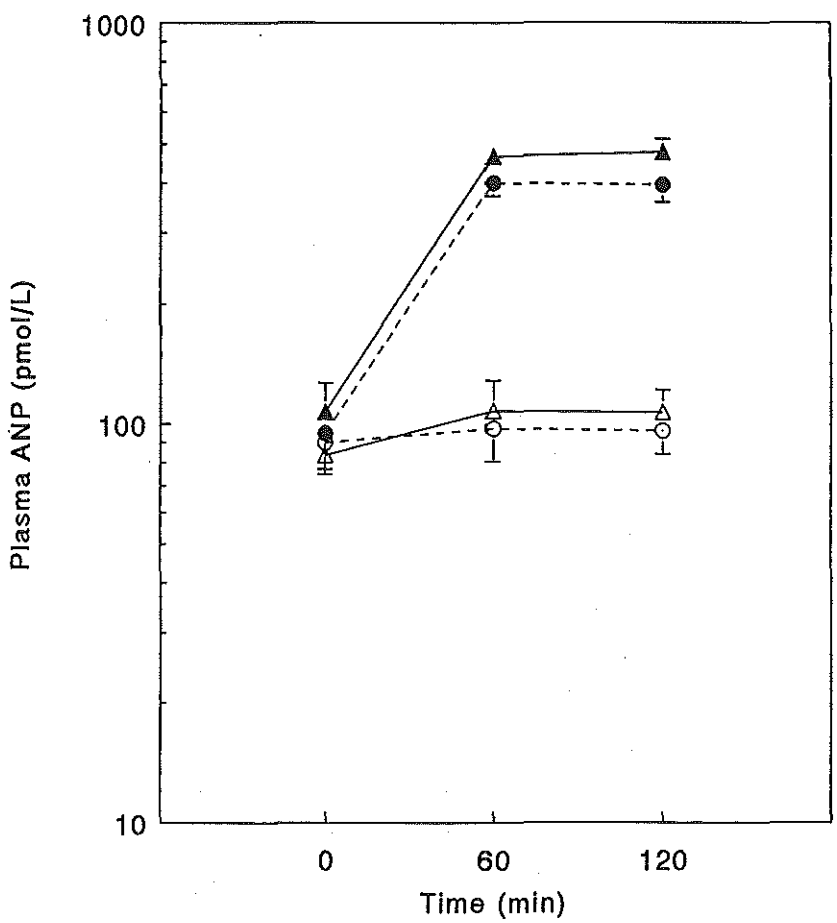
Procedures

Infusion protocol:

During a four week run-in period, all medication except insulin was discontinued and 24 hour sodium excretion was measured. One week before infusion all patients were placed on a diet containing the same amount of sodium as in the run-in period (as estimated by urinary sodium excretion). Subjects were admitted to the clinical research center at 8:00 AM after they had fasted from 12.00 PM on the previous day (except for ad libitum water intake). Subjects did not ingest any food until the study was completed. All individuals remained supine throughout the study. They were however allowed to stand in order to pass urine. At the start of the infusion of renal clearance markers all subjects received an oral water load of 20 ml/kg body weight. In order to achieve a steady state urine output, the urinary volume was replaced by drinking of tap water. Plastic cannulas were inserted into an antecubital vein of each arm. Insulin, dextrans and radiolabeled clearance markers were infused in one arm, while blood samples were drawn from the other arm.
After one hour equilibration the bladder was emptied by voiding. Urine was collected during six carefully timed 30 minute periods, the first two (from -60 to -30 min and from -30 to 0 min) serving as baseline periods (Fig. 1.). Mid-point plasma samples were taken and glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured in each of the clearance periods. The

mid-point plasma sample and urine collection during the second baseline period were also used to measure dextran concentrations. Following these baseline periods, human ANP (102-126) (Wy 47.663, Wyeth Research Laboratories, Philadelphia, Pennsylvania, USA) in vehicle (NaCl 0.9% containing 5% human serum albumin) or placebo (vehicle) was infused intravenously for 120 minutes at a dose of 0.01 μg/kg/min. Urine was collected during four 30 minute intervals (Fig. 1.). During the last interval (90 to 120 min.) a second dextran clearance measurement was done. At 60 minutes after the start of the infusion of ANP or vehicle, a single shot of ^{125}I-albumin was given (Fig.1.).

Fig. 2



In the subjects with insulin-dependent diabetes the usual morning dose of insulin was omitted and a constant infusion of insulin (1% of the daily dose per hour) in 5% glucose (50 mg/kg/min) was given from the start of the study. Subsequently blood glucose concentrations were measured at 30 minute intervals and kept between 4 and 8 mmol/L by intravenous injections of small doses of insulin or glucose 50%.

Clearance studies:

In order to determine GFR and ERPF, we measured the renal clearances of inulin (Inutest, Laevosan-Gesellschaft, Linz/Donau, Austria) and ^{131}I-orthoiodohippurate (Amersham, UK) respectively. The standard formula was used to calculate the clearance values. In order to achieve stable plasma concentrations of the clearance markers, the priming dose and sustaining infusion rate were adjusted to renal function as previously described [15]. This adjustment was based on prior estimation of renal function using the equation of Cockcroft and Gault [16].

Fractional clearance (F_{CL}) of macromolecules was calculated using the equation:

$$F_{CL} = [(U/P) / (U\text{-inulin}/P\text{-inulin})]$$

in which U and P are the urinary and plasma concentrations of the macromolecule. In each urine and plasma sample we also measured albumin, IgG and IgG_4 concentrations. The selectivity index (SI) was calculated as the clearance of IgG divided by that of albumin. In general, proteinuria is considered to be selective when SI is below 0.2 [17]. IgG and IgG_4 are of the same size but are differently charged at physiological pH. Therefore the clearance of IgG divided by that of IgG_4 can be used as a measure of charge selectivity (charge index, CI) [3]. The interpretation of the results of protein clearance measurements is complicated by tubular reabsorption of the proteins. In an attempt to correct for changes in reabsorption, we also measured the excretion of retinol binding protein (RBP).

Dextran clearances were performed as follows. At time -90 min. (Fig.1.), a 200 mg dose of dextran-10 (Promiten®, NPBI, Emmercompascuum, The Netherlands) was given in order to prevent anaphylactic reactions to subsequent doses. After ten minutes, 50 mg/kg dextran-70 (Macrodex®) was given in a 10 minute infusion, followed by a constant infusion of dextran-40 (Rheomacrodex®) at 100

mg/min, which was continued throughout the whole observation period.
Plasma volume and the transcapillary escape rate of albumin were calculated from ^{125}I counts in plasma samples taken 10, 15, 20, 30, 45 and 60 minutes after the injection of ^{125}I-albumin, as described by Parving et al. [18]. To correct for the higher baseline levels on the second day of study, a larger dose of ^{125}I-albumin was used (296 vs 148 kBq).

Analytical methods

Blood pressure was determined at 5 minute intervals, with an oscillometric device (Accutorr, Datascope Corp, Paramus, New Jersey, USA). The mean of the last three readings in each study period was used for analysis. Inulin concentrations were measured using the resorcinol assay [19]. Enzyme linked immunosorbent assays (ELISA) were developed for the determination of albumin, IgG and IgG_4 in plasma and urine and for measurement of urinary RBP. In brief, Maxisorb test plates (Nunc, Roshilde, Denmark) were coated with a monoclonal antibody directed against either albumin (A001), IgG (MH16-01-M06), IgG_4 (MH164-1-M05) or RBP (A040). The antibodies were all obtained from the Central Laboratory of Blood Transfusion, Amsterdam, The Netherlands. After overnight incubation with sufficiently diluted samples the plates were thoroughly washed, and a second, alkaline phosphatase labelled, monoclonal antibody against either albumin (P356), IgG(P214), or RBP (P304; Dakopatts, Glostrup, Denmark) was added. After washing the plates were incubated with substrate (Enzymun-test, Boehringer, Mannheim, Germany). Reference serum supplied by the Central Laboratory of Blood Transfusion was used as the standard. The sensitivity of the assays was 1 ng/ml. Interassay coefficients of variation were 5 to 7 % in ten subsequent assays.
Dextran was assayed in protein-free filtrates of plasma and urine (trichloric acetic acid 40%). These filtrates were separated into narrow fractions by gel permeation chromatography using the method described by Granath et al. [20]. A sephacryl S-300 column (Pharmacia, Upsala, Sweden) of 180 ml bed-volume and 90 cm in length was used. The eluent was a 0.01 M Tris buffer at pH 7.0 with 0.15 M NaCl containing 1 mM EDTA. Blue dextran (Mw 2,000,000 D) was used to determine void volume (V_o), and the column was calibrated using dextran T10, T40 and T70 (Pharmacia). The fractional volume available to the solute (K_{AV})

was calculated from:

$$K_{AV} = (V_e - V_o)/(V_t - V_o)$$

where V_e is the volume necessary to displace a solute from the column, and V_t is the total bed-volume of the gel column. Effective molecular radii for the individual dextran fractions were calculated from K_{AV}. After gel permeation chromatography, the eluate fractions were assayed for dextran using the anthrone method of Scott and Melvin [21].

The colloid osmotic pressure in the afferent arteriole (π_a) was assumed to be equal to systemic colloid osmotic pressure, and was calculated from plasma protein concentrations using the formula of Landis and Pappenheimer [22].

Plasma ANP levels were determined using a commercially available radioimmunoassay (Instar, Nijmegen, The Netherlands) after a previous extraction step using C-18 Seppak cartridges [23]. The normal range for this assay is 66 to 96 pg/ml (n=40, mean ± 2SD).

Analysis of membrane pore structure

To analyze the size-selective properties of the glomerular barrier, we used a heteroporous model of the glomerular capillary wall, as described by Deen et al [24]. This model has been shown to provide a satisfactory representation of dextran sieving. In this model the major portion of the capillary wall is perforated by restrictive cylindrical pores of identical radius (r_0). In addition, this model assumes a parallel 'shunt pathway' that does not discriminate on the basis of dextran size, and through which a small fraction (ω) of the filtrate volume passes. The size of this fraction is not merely dependent on the properties of the capillary wall but also on intracapillary oncotic pressure. Therefore a quantity is introduced that is closely related to ω but is independent of oncotic pressure and therefore characteristic of the membrane *per se*. This quantity (ω_0) is the fraction of the volume flux that would pass through the shunt if plasma proteins were absent [24]. The membrane barrier to dextrans is fully characterised by r_0, ω_0 and K_f, where K_f is the product of effective hydraulic permeability and glomerular capillary surface area. These variables can be estimated using curve fitting techniques. In order to make these calculations an estimate of the transmembrane hydraulic pressure difference (ΔP) is required. Since ΔP cannot

be measured directly in humans, a value must be assumed in order to calculate the basement membrane parameters. In normal humans ΔP is predicted to be close to 35 mmHg [25]. We therefore calculated intrinsic basement membrane parameters using a ΔP of 35 mmHg. Since experimental studies indicate that ANP may increase ΔP [26] and ACE inhibition may lower ΔP [27], we calculated basement membrane parameters using several different levels of ΔP (30, 35 and 40 mmHg). We have no direct measurements of ΔP in humans. This however, does not seriously hamper the use of dextran sieving as a measure of permselectivity because the calculation of basement membrane characteristics is relatively insensitive to changes in filtration pressure [24]. Thus, although the calculated changes in basement membrane parameters may not be exact, this method does give semi-quantitative information on the direction of such changes.

Statistical analysis

Baseline data, before and after enalapril treatment, were compared using a one-way ANOVA. When this yielded a significant F value, data were analyzed by the Student Neumann Keuls (SNK) test. Response to treatment was evaluated using a two-way ANOVA with repeated measures comparing the effect of ANP to that of vehicle. Differences in the effect of ANP before and after enalapril were evaluated by means of the paired Students t-test, comparing the total area under the curve (ANP minus vehicle). Dextran data and basement membrane characteristics were compared using one-way ANOVA and SNK tests. Urinary excretion data were not normally distributed and were therefore log-transformed before analysis. All calculations were performed using the SPSSPC+ statistical software package. Data are expressed as means ± SEM.

Results

The effect of ACE inhibition on plasma ANP levels.

The plasma levels of ANP in patients with diabetes and healthy control subjects were not significantly different at baseline and were well within our normal range. Treatment with enalapril did not significantly alter ANP levels before

infusion. Maximal plasma ANP levels achieved during the infusion of human ANP (102-126) were not affected by pretreatment with ACE inhibition (Fig. 2).

Effect of ACE inhibition on the glomerular filtration of water, sodium and proteins.

Treatment with an ACE inhibitor did not affect steady state urine and sodium outputs in patients with diabetes. ANP increased the excretion of both water and sodium by 64 and 107% respectively. No significant difference in the effects of ANP on water and sodium excretion was observed before and during enalapril (+35% and +83% respectively).

ANP infusion did not alter glomerular filtration rate (GFR), effective renal plasma flow (ERPF) and filtration fraction (FF)(Table 2), either before or during enalapril. ACE inhibition tended to increase baseline ERPF (603 ±53 to 661 ±55 ml/min), although this increase did not reach statistical significance. No significant changes occurred in GFR or FF.

In our patients with diabetes and microalbuminuria one month of ACE inhibition reduced the excretion rates of albumin and IgG by 38 and 41% respectively. In contrast to healthy subjects studied previously, ANP induced a marked increase in protein excretion in the diabetic subjects [8]. This proteinuric effect of ANP was not prevented by ACE inhibition (Fig 3), and the percentual increase in both albumin and IgG excretion was not significantly different after ACE-inhibition (+200% to 186 % and +163 to 140 % respectively). Excretion of IgG_4 in diabetic subjects increased during the infusion of ANP infusion, both before and during enalapril treatment. The charge selectivity of the proteinuria, calculated as charge index (CI), was not significantly altered by ANP infusion nor by ACE inhibition.

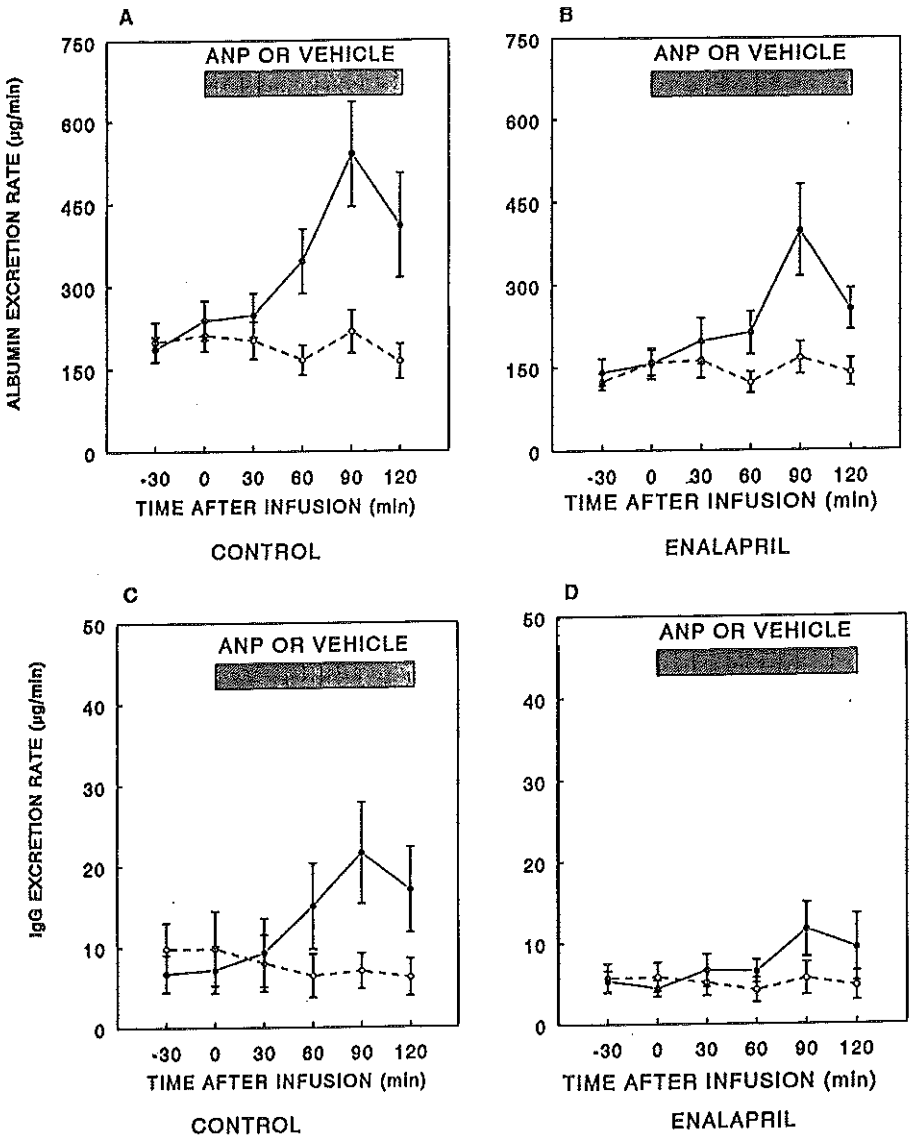
In our patients with microalbuminuric diabetes, size selectivity of the proteinuria (calculated as size-index, SI), at the onset of the infusion period, was not significantly different from normal individuals [8]. ACE inhibition did not alter baseline selectivity index. During ANP infusion, SI remained unchanged. ACE inhibition did not change the response of SI to ANP infusion. The urinary excretion rates of retinol binding protein were not altered by ACE inhibition or the infusion of ANP.

Table 2

			BASELINE	ANP 0.01 μg/kg/min or VEHICLE			
				30 min.	60 min.	90 min.	120 min.
GFR (ml/min)	CONTROL	*vehicle*	114 ± 9.9	116 ± 10.1	106 ± 6.5	112 ± 13.0	112 ± 10.9
		ANP	116 ± 7.7	115 ± 11.9	114 ± 10.0	122 ± 14.0	120 ± 9.7
	ENALAPRIL	*vehicle*	122 ± 8.7	116 ± 10.7	105 ± 4.8	118 ± 8.1	120 ± 8.7
		ANP	123 ± 13.9	125 ± 10.1	99 ± 11.9	117 ± 9.3	114 ± 7.4
ERPF (ml/min)	CONTROL	*vehicle*	603 ± 52.5	590 ± 50.2	535 ± 35.1	563 ± 54	553 ± 52.5
		ANP	617 ± 37.9	616 ± 49.4	552 ± 38.0	544 ± 53.3	619 ± 61.2
	ENALAPRIL	*vehicle*	661 ± 54.5	643 ± 74.4	579 ± 44.8	649 ± 46.4	674 ± 59.8
		ANP	671 ± 69.6	727 ± 72.7	540 ± 60.6	598 ± 65.4	603 ± 59.4
FF	CONTROL	*vehicle*	0.19 ± 0.02	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.21 ± 0.01
		ANP	0.19 ± 0.02	0.19 ± 0.01	0.21 ± 0.01	0.22 ± 0.01	0.20 ± 0.01
	ENALAPRIL	*vehicle*	0.18 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	0.19 ± 0.01
		ANP	0.18 ± 0.01	0.17 ± 0.01	0.19 ± 0.01	0.20 ± 0.02	0.19 ± 0.01

No significant differences where found between ANP and vehicle, or between enalapril and control.

Fig. 3

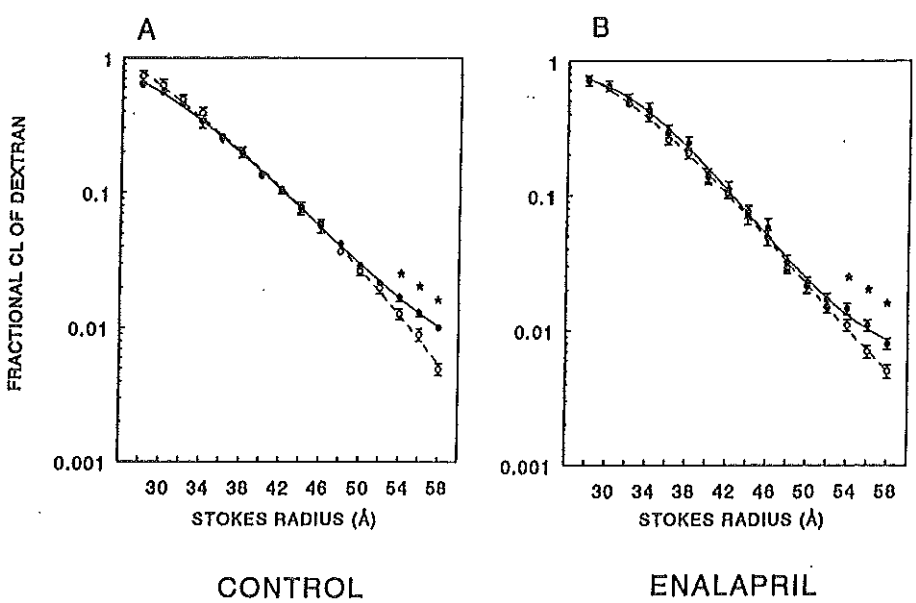

A
B
C
D
ANP OR VEHICLE
ALBUMIN EXCRETION RATE (µg/min)
IgG EXCRETION RATE (µg/min)
750
600
450
300
150
0
50
40
30
20
10
-30
0
30
60
90
120
TIME AFTER INFUSION (min)
CONTROL
ENALAPRIL

Effect of ACE inhibition on dextran clearances and glomerular permeability characteristics.

The previously described differences in dextran sieving behaviour between diabetic and healthy subjects [8], consisting of a significant depression of fractional clearances of dextrans with molecular radii of 36 Å and below, were also found during ACE inhibition (Fig. 4). Infusion of ANP induced a significant increase in the fractional clearance of dextran molecules 54 Å and larger. This increase remained also present during enalapril.

Calculations indicated that glomerular permeability in the diabetic state differed from normal in that the hydraulic permeability (K_f) was depressed (Table 3). In patients with diabetes, infusion of ANP induced a marked increase in the "shunt-flow" parameter ω_0. ACE inhibition did not significantly alter the permeability characteristics in our subjects. The ANP-induced increase in "shunt-flow" observed in diabetic subjects persisted during enalapril treatment.

Fig. 4



Effect of ACE inhibition on blood pressure, plasma volume and the transcapillary escape rate of albumin.

In our patients, blood pressure was not significantly altered during treatment with enalapril (systolic pressure 139 ±6.6 before enalapril vs. 133 ±5.1 mmHg after, diastolic pressure 78 ±4.5 vs 75 ±2.4 mmHg). ANP infusion did not alter blood pressure neither before nor after ACE inhibition.

ACE inhibition did not affect plasma volume in our diabetic subjects (3128 ±588 before vs. 3133 ±717 ml during ACE inhibition). Before ACE inhibition, ANP significantly increased the hematocrit (0.38 ±0.03 to 0.41 ±0.02 L/L; $p < 0.05$). Following ACE inhibition baseline hematocrit was lower and the increase in hematocrit in response to ANP failed to reach statistical significance (0.35 ±0.01 to 0.37 ±0.02).

Before ACE inhibition, the transcapillary escape rate of albumin (TER_{alb}) was significantly higher in diabetic subjects than in 10 healthy subjects studied previously (9.2 ±1.4 vs. 5.4 ±2.2 %/h) [28]. ANP infusion increased the TER_{alb} both before and during ACE inhibition (from 9.2 ±0.5 to 13.5 ±0.9 %/h and from 8.9 ±0.7 to 12.8 %/h respectively).

Discussion

The present study demonstrates that treatment with an angiotensin converting enzyme (ACE) inhibitor markedly reduces protein excretion in patients with microalbuminuric diabetes mellitus. This is not surprising as previous studies have shown an antiproteinuric effect of ACE inhibition in all phases of diabetic nephropathy [9][10]. Interestingly, we observed that, despite reducing the level of protein excretion, ACE inhibition did not alter the response of glomerular permeability to the infusion of atrial natriuretic peptide (ANP). Thus, the subclinical defect in glomerular barrier function present in diabetes, which becomes evident during ANP infusion, is not abolished by enalapril treatment. This defect is characterised by an increase in the fractional clearances of large dextrans during ANP infusion [8], suggesting an increase in flow through large unselective "shunt" pores permeating the filtration barrier [24]. In healthy subjects, ANP does not induce alterations in glomerular permeability [8].

Table 3

		ΔP	K_f	r_o	ω_o
		mmHg	ml.min^{-1}.mmHg	Å	x 10^{-3}
NORMALS		35	13.3 ± 0.9	56.9 ± 0.01	1.4 ± 0.1
CONTROL	*vehicle*	35	9.8 ± 1.3 [1]	55.7 ± 0.01	1.8 ± 0.3
	ANP	35	10.1 ± 1.2 [1]	55.9 ± 0.01	6.1 ± 0.4 [1,2]
		40	9.1 ± 1.1 [1]	55.9 ± 0.01	6.4 ± 0.4 [1,2]
ENALAPRIL	*vehicle*	30	11.1 ± 1.1	55.8 ± 0.01	1.5 ± 0.3
		35	10.2 ± 1.1	55.9 ± 0.01	1.7 ± 0.4
	ANP	30	10.9 ± 1.0	56.0 ± 0.01	5.4 ± 0.3 [2]
		35	10.1 ± 1.2	56.1 ± 0.01	5.7 ± 0.3 [2]
		40	9.1 ± 1.1	56.1 ± 0.01	5.9 ± 0.3 [2]

[1] $p<0.05$ vs normals, [2] $p<0.05$ vs vehicle.

In diabetes several abnormalities of the glomerular filtration barrier exist which may be related to the observed susceptibility to the effects of ANP. Increased nonenzymatic glycation of type IV collagen may cause unphysiological cross-linking which can lead to alteration of the mesh structure of the glomerular basement membrane (GBM) [29]. Loss of heparan sulphate proteoglycan (HSPG) leads to reduction of the negative charge content of the GBM [30], which may alter glomerular permeability, either through disruption of GBM microstructure [31][32], or as a result of detachment of glomerular epithelial cells from the GBM [33]. Furthermore glomerular hypertrophy, which is known to occur in diabetic nephropathy, may result in areas of the peripheral glomerular capillary that are no longer covered by epithelial cells and that permit protein trafficking [34]. Each of these abnormalities would make the glomerular filtration barrier more vulnerable to ANP-induced hemodynamic stress i.e., increased ΔP due to afferent renal vasodilation and efferent vasoconstriction [35].

manipulations, other than ANP, to increase proteinuria have yielded conflicting results. The increase in albumin excretion during exercise was reduced [36], whereas increased protein excretion during aminoacid infusion was not affected [37]. These studies however, concern the acute effects of ACE inhibition which are most likely related to changes in renal hemodynamics. The time period of these studies is probably too short for the development of structural changes in the filtration barrier. For this reason we studied our subjects after a longer period of treatment. Even so, this period may still not be long enough for ACE inhibition to have its full effect on GBM structure and function. A more recent study indicates that the maximal effect of converting enzyme inhibition on proteinuria is reached after a period of three months [38].

We have previously shown that the effect of ANP on the transcapillary passage of proteins is not restricted to the glomerulus, but may occur throughout the circulation [28]. Although ACE inhibition can block the effect of ANP on hematocrit in rats [39], in our diabetic subjects we found no effect of ACE inhibition on the ANP mediated increase in the transcapillary escape rate of albumin.

We conclude that ACE inhibition during one month does not correct the capillary barrier function defect that is unmasked by ANP infusion.

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Chapter 9

Summary and conclusions

Increased protein excretion in the urine due to a reduction in glomerular barrier function generally heralds loss of renal function.
The studies presented in this thesis were initiated to gain more knowledge concerning the way in which various diseases affect glomerular barrier function.

In **chapter 1** a brief introduction is presented to the anatomical and functional characteristics of the glomerular filtration barrier, both in health and disease. The effects of various physiological and pharmacological manoeuvres on the glomerular filtration of macromolecules are also discussed.

In **chapter 2** we describe a method by which glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) can be measured accurately, even when renal function is severely impaired. Using a simple (one compartment) pharmacokinetic model we were able to achieve steady state concentrations of the radiolabelled tracers within a 90-minute period. These studies also indicated that in patients with diabetes mellitus estimation of GFR using Cockcroft's equation

severely underestimates GFR when measured by ^{125}I-iothalamate clearance.

In **chapter 3** we present our findings in the first 17 patients with refractory nephrotic syndrome due to various forms of glomerular disease that were treated with cyclosporine A (CsA) at our center. The effect of CsA on protein excretion varied markedly in the different disease groups. In an attempt to study the mechanism of the CsA-induced reduction in protein excretion we calculated the fractional clearances of total protein. In three patients with non-immunologically mediated nephrotic syndrome due to Alport's syndrome CsA reduced proteinuria, but did not affect the fractional clearance of protein. We interpreted these findings as an indication that in these patients the reduction in proteinuria during cyclosporine was secondary to its effects on glomerular hemodynamics. In both minimal change disease MCD) and membranous glomerulopathy (MG) the fractional clearance of protein was significantly reduced by CsA.
We concluded that in MCD and MG the immunological effect of CsA may be of importance in its effect on proteinuria.

These findings lead us to the more detailed study of the effects of CsA on glomerular barrier function described in **chapter 4**. In order to dissociate perturbations in glomerular hemodynamics from alterations in the permeability of the glomerular filtration barrier we used the fractional clearances of graded dextrans with subsequent calculations based on the "isoporous plus shunt" model devised by Deen and coworkers. In this way we were able to show that in MCD CsA increased the hydraulic permeability coefficient (K_f) towards normal, but did not affect size-selectivity. The impressive reduction in proteinuria that was observed in these patients during CsA treatment was the result of a significant increase in glomerular charge selectivity (estimated by dividing the clearance of total IgG by that of the more anionic IgG_4 fraction). In MG CsA dramatically increased glomerular size-selectivity as reflected by a fourfold reduction in flow through the large unrestrictive shunt-pores.
From these findings we concluded that in MCD and MG CsA alters the permeability of the filtration barrier independently of its effect on glomerular

hemodynamics.

Using similar clearance techniques we studied the reversibility of the detrimental effects of CsA on the glomerular barrier function following renal transplantation. These findings are presented in **chapter 5**. Despite a marked improvement in both ERPF and GFR that resulted from discontinuing CsA in renal transplant recipients (i.e. conversion to azathioprine; AZA) with moderate renal failure, no changes in dextran sieving were observed. However, correction of these dextran data for the observed increase in glomerular perfusion, which would theoretically lower the fractional clearance of small dextrans, using Deen's model resulted in a trend towards increased K_f. This trend was offset when assuming an increase in ΔP due to reduced pre-glomerular vasoconstriction following conversion to AZA.
We concluded that conversion from CsA to AZA did not significantly affect glomerular barrier function. The observed improvement in renal function is likely to be due to an increase in glomerular capillary plasma flow, although an increase in K_f or ΔP could also participate.

In **chapter 6** we report on the effects of infusion with ANP in patients with nephrotic syndrome. The most striking finding in these studies was the effect of ANP on capillary permeability. Infusion of ANP increased the urinary excretion of both albumin and IgG. Furthermore, ANP induced hemoconcentration and loss of albumin from the vascular compartment that was not attributable to the excretion of albumin in the kidney. Although the mechanism by which ANP exerted its effect on the glomerular filtration of proteins was unclear, we felt that ANP-infusion might prove a useful tool in studying the glomerular filtration barrier.

We used ANP-infusion and dextran clearance techniques to probe the glomerular permeability in patients with diabetes mellitus and microalbuminuria, as described

in **chapter 7**. We were able to demonstrate that in diabetics baseline size-selectivity was not different from healthy control subjects. In contrast, ANP-infusion induced a significant loss of glomerular size-selectivity as indicated by an increase in shunt-flow (ω_0).
Thus ANP uncovered a derangement in size-selective glomerular barrier function in diabetic subjects in a phase that is otherwise characterised by charge-selective changes only. As ANP also increased the transcapillary escape rate of albumin this increased susceptibility could be part of a more generalized capillary abnormality.

In **chapter 8** we expand on these findings comparing ANP-infusions before and during ACE-inhibition. As ACE-inhibition has been shown to increase glomerular size-selectivity, we investigated whether 3 months of ACE-inhibition could correct the subclinical defect in glomerular barrier function that is unmasked by ANP infusion. Despite a 40% reduction in proteinuria, ACE-inhibition did not prevent the changes in dextran sieving induced by ANP.
Thus three months of ACE-inhibition did not correct these early abnormalities of the glomerular filtration barrier in diabetes mellitus.

Samenvatting

Een toegenomen eiwit uitscheiding in de urine (proteïnurie) ten gevolge van een toename in doorlaatbaarheid van de glomerulus (nefrotisch syndroom), die normaal functioneert als zeef, is meestal een voorteken van verlies van nierfunctie.
De studies beschreven in dit proefschrift werden opgezet om een beter inzicht te verkrijgen in de manier waarop diverse nierziekten de glomerulaire barrière beïnvloeden.

In **hoofdstuk 1** wordt een korte inleiding gegeven met betrekking tot de anatomische en functionele kenmerken van de glomerulaire barrière voor filtratie van eiwitten, zowel in de gezonde situatie als bij diverse nierziekten. Bovendien worden de effecten van diverse geneesmiddelen en fysiologische veranderingen op de glomerulaire doorlaatbaarheid voor eiwitten besproken.

In **hoofdstuk 2** beschrijven we een methode waarmee de glomerulaire filtratie snelheid (GFR) en de effectieve renale bloeddoorstroming (ERPF) nauwkeurig

kunnen worden gemeten, zelfs bij slechte nierfunctie. Met gebruikmaking van een eenvoudig (één compartiment) farmacokinetisch model was het mogelijk om binnen 90 minuten een stabiele bloed concentratie van de radioactieve test substanties te verkrijgen. Deze studie toonde ook aan dat bij patiënten met suikerziekte berekening van de GFR met behulp van de benaderingsformule van Cockcroft de GFR ernstig onderschat.

In **hoofdstuk 3** beschrijven wij de resultaten van de eerste 17 patiënten met een nefrotisch syndroom, niet reagerend op conventionele behandeling, die op onze afdeling werden behandeld door middel van remming van de natuurlijke afweer met behulp van Cyclosporine A (CsA). Het effect van CsA wisselde sterk per ziektebeeld. In een poging het mechanisme van de CsA-geïnduceerde vermindering van de eiwit uitscheiding te onderzoeken corrigeerden we de eiwit uitscheiding voor de filtratie van water. In drie patiënten met de ziekte van Alport, waarin het immuunapparaat waarschijnlijk geen rol speelt, verminderde CsA de eiwit uitscheiding, doch na correctie bleek deze ongewijzigd. Deze bevinding interpreteerden wij als een aanwijzing dat CsA in deze patiënten de eiwit uitscheiding deed afnemen door afname van de glomerulus filtratie. In zowel "minimal change disease" (MCD) als in "membranous glomerulopathy" (MG) trad wel een afname van de gecorrigeerde eiwit uitscheiding op.
Wij concludeerden dat in MCD en MG het effect van CsA op het afweerapparaat een rol zou kunnen spelen bij de afname van de eiwit uitscheiding.

Deze bevindingen waren de aanzet tot de meer gedetailleerde studie naar de effecten van CsA op de glomerulaire barrière zoals beschreven in **hoofdstuk 4.** Teneinde de veranderingen in doorlaatbaarheid voor eiwitten te kunnen differentiëren van veranderingen in o.a. bloeddoorstroming en filtratie van water (hemodynamiek) gebruikten wij de gecorrigeerde uitscheidingen van suiker-polymeren met een uiteenlopende diameter (dextranen) gevolgd door berekeningen met behulp van het mathematische model ontwikkeld door Deen en medewerkers. Hiermee konden we aantonen dat CsA in MCD de doorlaatbaarheid voor water (K_f) normaliseerde, maar geen effect had op de

selectiviteit van de nier op basis van grootte van de moleculen. De indrukwekkende afname van de proteïnurie bij deze patiënten bleek het gevolg van een toename in de selectiviteit voor de lading van moleculen. In MG trad ten gevolge van CsA wel een dramatische verbetering op van de grootte-selectiviteit. Uit deze bevindingen concludeerden wij dat in MCD en MG het effect van CsA op de glomerulaire barrière onafhankelijk is van effecten op de glomerulaire hemodynamiek.

Met behulp van soortgelijke methoden onderzochten wij de omkeerbaarheid van de nadelige effecten van CsA, dat zelf ook nierschade kan veroorzaken, op de glomerulaire barrière na niertransplantatie. Deze gegevens worden beschreven in **hoofdstuk 5**. Ondanks een duidelijke verbetering van zowel ERPF als GFR ten gevolge van het staken van CsA bij deze patiënten (d.w.z. dat de behandeling werd voortgezet met een ander geneesmiddel, te weten azathioprine; AZA) werden geen veranderingen waargenomen in de uitscheidingen van dextranen. Als we deze gegevens echter corrigeren voor de gemeten toename van ERPF en GFR met behulp van de methode van Deen zou er een toename plaats gevonden kunnen hebben van de doorlaatbaarheid voor water (K_f).
Wij concludeerden dat staken van CsA geen evidente invloed heeft op de glomerulaire barrière. De verbeterde nierfunctie is waarschijnlijk vooral het effect van toegenomen bloeddoorstroming, hoewel toename van de K_f of verhoging van de druk in de glomerulus ook een rol zouden kunnen spelen.

In **hoofdstuk 6** beschrijven we het effect van toediening van atriaal natriuretisch peptide (ANP), een door het hart afgegeven eiwit dat o.a. de zout uitscheiding in de nier sterk doet toenemen, bij patiënten met het nefrotisch syndroom. Het belangrijkste effect van ANP bleek een toename van de doorlaatbaarheid van de vaatwand voor eiwitten. Dit had zowel toename van de eiwit uitscheiding als verlies van eiwit uit de bloedbaan naar het losmazig bindweefsel tot gevolg. Hoewel het niet duidelijk was langs welk mechanisme ANP de proteïnurie deed toenemen leek toediening van ANP ons potentieel van nut om de glomerulaire filtratie barrière te onderzoeken.

Wij gebruikten ANP infusie en de dextran technieken om de glomerulaire doorlaatbaarheid voor eiwitten te onderzoeken bij patiënten met suikerziekte en microalbuminurie (een kleine toename in de eiwit uitscheiding, mogelijk ten gevolge van afname van de ladings-selectiviteit, die waarschijnlijk voorspellend is voor toekomstige achteruitgang van de nierfunctie), zoals beschreven in **hoofdstuk 7**. In tegenstelling tot gezonde controle personen veroorzaakte ANP bij de patiënten met suikerziekte een duidelijke afname van de selectiviteit op basis van grootte, die voor toediening van ANP niet afwijkend was. Zodoende was het mogelijk met behulp van ANP een afwijking aan te tonen van de grootte-selectiviteit van de glomerulaire barrière in een fase van de ziekte die onder normale omstandigheden gekenmerkt wordt door een afwijking van de ladings-selectiviteit. Aangezien ANP ook de ontsnapping snelheid van eiwit uit de bloedbaan deed toenemen zou dit kunnen wijzen op een meer gegeneraliseerde afwijking van de vaatwand.

In **hoofdstuk 8** herhaalden we deze experimenten tijdens behandeling met een bloeddruk verlagend middel (ACE-remmer) waarvan bekend is dat het de eiwit uitscheiding in sommige situaties kan verminderen. Ondanks een afname van de eiwit uitscheiding met 40% na behandeling gedurende drie maanden met een ACE-remmer werd het effect van ANP op de glomerulaire doorlaatbaarheid voor eiwitten en dextranen niet voorkomen.
Zodoende lijkt behandeling met ACE-remming gedurende drie maanden de vroege afwijkingen van de glomerulaire barrière bij suikerziekte niet op te heffen.

Curriculum vitae

De schrijver van dit proefschrift werd op 7 mei 1958 geboren te Willemstad Curaçao. Na het behalen van het Atheneum-B diploma aan het Eerste Vrijzinnig Christelijk Lyceum te 's-Gravenhage werd in 1976 aangevangen met de studie geneeskunde aan de Rijks Universiteit Leiden, alwaar in 1983 het artsexamen werd afgelegd. Van 1983 tot 1985 was hij werkzaam als arts-assistent Inwendige Geneeskunde in het Medisch Centrum Alkmaar. Aansluitend werd begonnen met de opleiding tot internist op de afdeling Interne Geneeskunde I, Academisch Ziekenhuis Rotterdam-Dijkzigt, opleider Prof. Dr. M.A.D.H. Schalekamp, waarna hij op 1 april 1990 werd ingeschreven in het specialisten register. Vervolgens werd hij opgeleid tot nefroloog (opleider Prof. Dr. W. Weimar). In 1993 werd hij als zodanig geregistreerd. Sedertdien is hij als staflid verbonden aan de afdeling Interne I van het Dijkzigt Ziekenhuis.

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