Dynamic renal function testing by compartmental analysis: assessment of renal functional reserve in essential hypertension

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Abstract

Background. In essential hypertension, acute haemodynamic changes due to dietary protein load cause patterns of acute changes in renal function that are fundamentally different from changes in normal controls.

Methods. Renal clearances of sinistrin, an inulin-like polyfructosan, and p-aminohippurate were determined before and after protein ingestion. These tests were performed in healthy controls and in patients with essential hypertension (mean arterial pressure of 112 ± 2 mmHg, age, 52 ± 2 years; mean ± SEM) within a washout period, and after long-term treatment with carvedilol and fosinopril, respectively.

Results. In 15 healthy volunteers, protein ingestion increased glomerular filtration rate (GFR) from 110.3 ± 3.6 to 120.6 ± 4.4 ml/min (P = 0.0006; two-tailed pairwise t-test). In contrast, it led to an acute decrease in GFR in 16 hypertensive patients, from 111.8 ± 2.9 to 103.6 ± 3.3 ml/min (P = 0.0010). The eight patients who were randomized to receive carvedilol improved in their renal response to protein (GFR increased from 101.4 ± 6.4 to 107.1 ± 5.4 ml/min; P = 0.04), whereas the eight other patients randomized to receive fosinopril exhibited no change in GFR (final value 105 ± 4.9 ml/min). In the patients, the acute shifts in renal plasma flows were not significant. Mean arterial blood pressure of the patients decreased from 112 ± 2 to 100 ± 3 mmHg (P = 0.0015).

Conclusions. In essential hypertension an acute protein load induces a decrease in GFR that may normalize under antihypertensive treatment. The acute changes in GFR can be reliably monitored by the here-described compartmental analysis method of renal functional reserve.

Keywords: dietary protein load; effective renal plasma flow; essential hypertension; glomerular filtration rate; renal functional reserve

Introduction

Sensitive clinical methods for detecting early changes in renal vascular reactivity of patients with essential hypertension have been lacking to date [1]. Because of possible hyperfiltration [2], single clearance measurements cannot detect vascular changes, which may be induced by hypertension or reversed by antihypertensive treatment. Therefore, dynamic renal function tests, which consist of two consecutive kinetic clearance measurements before and after a dietary protein load, have been tried previously in patients at risk for hypertensive renal damage in order to assess the extent of renal vascular impairment [3]. In healthy subjects, increases in glomerular filtration rate (GFR) after protein ingestion have been uniformly observed [4], but in patients with essential hypertension most authors have found ‘blunted increases’ in GFR after dietary stresses [5]. This so-called ‘blunted increase’ in GFR was originally thought to be due to an increase in intraglomerular pressure. However, the alleged increase in GFR in response to protein ingestion was not accompanied by a change in albumin excretion, and there was lack of dynamic GFR response after administration of ACE inhibitors.

These contradictory results suggest that the earlier dynamic test methods that described qualitatively normal increases in GFR after amino acid stimulation were probably not sensitive enough. Stationary creatinine levels cannot reveal short-term changes in clearance. Traditional steady-state methods of GFR determination [6] are inappropriate for acute evaluation of dynamic changes in kidney function because of their mathematical naivété. The ‘gold standard’ in renal clearance determinations formerly consisted of relating the urinary elimination rate to the corresponding plasma concentration level of an excreted marker [7]. ‘Constant infusion’ techniques were employed for
clearance determinations. Evaluation was done by forming the ratio of infusion or elimination rate and the steady-state marker level. These methods required equilibration of marker concentrations between the different compartments, and therefore between marker influx and elimination. Since there is necessarily a generally unknown delay between the changes of plasma and urinary signals, constant infusion methods for calculation of renal clearance are correct only over a long experimental time horizon [8]. However, in dynamic renal function testing the experimental time horizons are necessarily limited for practical reasons.

A mathematical extension for the description of time-dependent non-steady-state processes is given by the superposition of exponential functions. These empirical models implicitly represent processes of marker distribution and elimination in one or more compartments [9]. However, only the concentration profile in the so-called central compartment, i.e. the blood compartment, is studied after a single injection of a marker bolus. Furthermore, only situations with initial marker concentrations of zero before marker application are considered.

In a model for dynamic renal function testing, however, marker amounts that remain in the extracellular space from the first kinetic experiment have to be taken into account for evaluation in an immediately following experiment. In order to take into account non-zero initial marker concentrations, a more flexible approach was employed that avoided the stereotypical transference of the traditional renal clearance technique by adapting the basic pharmacokinetic model [10] to experimental non-steady-state data gained within feasibly short experimental time horizons. The model describes the distribution of injected marker amounts in the extracellular space as represented by a central and a peripheral compartment, and the simultaneous elimination process from the central compartment [11].

The system constants of the model equations are identified in a search algorithm for the calculation of clearance and distribution parameters of an individual patient [12].

We investigated the suitability of system identification as applied to a two-compartment model for quantitative assessment of protein-rich meals on renal function. In order to evaluate the dynamic renal function testing procedure we studied the dynamic test response defined as the immediate change in clearance following the dietary protein load in healthy controls and in some hypertensive patients. In addition, two groups of hypertensive patients, which were formed randomly from the larger group, were tested prior to and following long-term treatment with the β-adrenoceptor blocker carvedilol and the angiotensin converting enzyme (ACE)-inhibitor fosinopril, respectively. The aim of our study was to test whether or not the dynamic test method can detect alterations in renal vascular status in patients with essential hypertension by determination of acute renal functional change following protein ingestion [13].

Subjects and methods

The subjects included 15 healthy controls (four male, 11 female, mean age 44.1 ± 2.3 years, mean arterial pressure MAP = 1/3 × (systolic pressure + 2 × diastolic pressure) 93.7 ± 3 mmHg), and 16 hypertensive patients (five male, 11 female, mean age 52.3 ± 1.6 years, MAP 112 ± 2 mmHg, mean duration of hypertension 8.4 ± 3.1 years; data given as mean ± SEM). Eight hypertensive patients were randomly chosen for 6 months treatment with the β-adrenoceptor-blocker carvedilol. The other eight patients were chosen for 6 months treatment with the ACE inhibitor fosinopril. All subjects were studied first after a 2-week washout period without antihypertensive drugs, and again after 6 months of treatment with either carvedilol or fosinopril. All subjects had a normal serum creatinine level, normal creatinine clearance, no proteinuria, and no history of any renal disease.

The determination of renal functional reserve requires the stimulation of renal function by amino acid infusion or a protein-rich meal [14]. We chose an artificially protein-rich meal for protein supplementation in order to avoid the local pain and phlebitis noted in preliminary experiments using peripheral intravenous administration of amino acids. The time-span chosen for protein ingestion was motivated by previous studies [15] in healthy humans that showed that GFR changes from one constant level to another approximately 90 min after ingestion of amino acids. In these studies the new GFR remained constant for at least 3 h.

Subjects were instructed to eat a low-protein diet for 2 days before the examination. On the morning of the test they had been fasting since midnight. Every hour the test subjects were given 4 ml of water/kg body weight, and remained recumbent throughout the entire study. The test meal (1.0 g protein/kg body weight) was given at the end of the first clearance determination. The second clearance determination was made 90 min after the end of the first determination. For a 70 kg subject, the meal consisted of 40 g of a commercial protein-rich liquid diet (Meritene®, Wander, Switzerland) containing l-arginine, supplemented by 30 g of protein powder (Powerplay®, Wander). For determination of GFR and effective renal plasma flow (ERPF), 2500 mg sinistrin (Inutest®, Fresenius Pharma Austria, Linz, Austria), an inulin-like polyfructosan, and PAH (Aminohippurate®, Merck & Co, West Point, PA, USA) in a dosage of 10 mg/kg body weight (minimal PAH dose 500 mg, maximal PAH dose 1000 mg) were given intravenously over 3 min.

Carvedilol was given in a dose of 25–50 mg/day. The dosage of fosinopril was 10–20 mg/day. Dosages of the drugs were then adjusted to achieve a desired MAP goal of 100 mmHg. The study was approved by the local ethics committee, and the subjects had given informed consent.

The evaluations of the dynamic renal function tests were performed by adapting the basic model of pharmacokinetics to the marker concentration profiles. The methods involved have been described previously [16,17]. All clearance estimates referred to 1.73 m² body surface area. Paired and unpaired Student’s two-tailed t-tests were used for comparison of group mean values. All means are given with their SEM. The statistical evaluations were done by means of the spreadsheet program Microsoft Excel (Version 5.0).

Results

The temporal concentration profile of sinistrin in a hypertensive patient during the initial dynamic study
is shown in Figure 1. It illustrates the studies and evaluations done on all tested subjects.

Figure 2 shows the MAP in the normotensive controls, the hypertensive patients during the washout period, and the same hypertensive patients after long-term antihypertensive treatment. There is a long-term decrease in blood pressure, but no acute change in MAP due to the dietary stimulus during the dynamic renal function studies.

Figures 3 to 7 illustrate that the method of dynamic renal function testing allows the monitoring of long-term pathophysiological and treatment-dependent changes in renal functional responses to dietary stimuli. The essence of this is shown in Figure 3 and it can be seen that in normotensive controls the mean GFR increases upon protein stimulation whereas in hypertensive patients it decreases. There is a tendency towards normalization of the dynamic test response to protein stimulation in the hypertensive patients after long-term antihypertensive treatment due to the effect of carvedilol.

In Figure 4 the mean ERPF for normotensive con-

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**Fig. 1.** Sinistrin concentration profile in a hypertensive patient before and after the oral protein load during the initial washout phase. Experimental data points are represented by solid squares. The solid line represents the model-fitted curve.

**Fig. 2.** MAP during baseline and test clearance experiments, i.e. before and after protein ingestion, respectively. NT, normotensive controls ($n=15$); HT0, hypertensive patients during washout phase ($n=16$); HTt, same hypertensive patients after long-term antihypertensive treatment ($n=16$). Error bars denote SEM. Differences in corresponding MAPs between groups NT, HT0 and HTt are significant ($P<0.05$). Differences between baseline and test MAPs are not significant.
controls increases upon protein stimulation, but in hypertensive patients it remains the same. There is a tendency towards normalization of the dynamic test response to protein stimulation in the hypertensive patients after long-term antihypertensive treatment. A striking feature of these results is the large difference in ERPF between normotensive subjects and hypertensive patients. This difference does not disappear with long-term antihypertensive treatment. Both antihypertensive drugs, carvedilol and fosinopril, led to the same decrease in MAP as illustrated in Figure 2 for the total group of hypertensive patients after long-term treatment.

As Figures 5 and 6 indicate, both subgroups of hypertensive patients initially show about the same decrease in GFR upon protein stimulation. Regarding the renal functional response after long-term treatment, however, there seems to be a fundamental difference in the actions of carvedilol and fosinopril. Figure 5 shows that after long-term treatment with carvedilol there is an increase in GFR upon protein stimulation. A restoration of the response exhibited by the normal controls is achieved by the treatment. Figure 6 shows that after long-term treatment with fosinopril, GFR before and after protein stimulation remains the same. Thus it seems that if there was any response at all, it
was only a partial restoration of the normal haemodynamic response.

Since dynamic processes are generally more appropriately characterized by fractional increases or decreases, and since GFR is determined more directly than ERPF, which is only approximated by the clearance of PAH, Figure 7 summarizes and accentuates the findings illustrated in Figures 3, 5 and 6 by means of the relative change in GFR, i.e. the difference between GFR before and after protein stimulation, divided by GFR before protein stimulation. \( \Delta \text{GFR}/\text{GFR} \) is referred to as renal functional reserve (RFR).

The renal vascular resistance (RVR) for the normotensive controls, the patients with essential hypertension, the patients treated with carvedilol, and the patients treated with fosinopril is shown in Figure 8. RVR was calculated by the formula of Gomez: 

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\text{RVR} = \left(\frac{\text{MAP} - 10}{\text{ERPF}}\right) \times 60 \times 1322 \times (1 - \text{Haematocrit}) \quad \text{(dyn/cm}^2\text{)/(ml/s)}
\]

Upon comparison of Figure 7 with Figure 8, acute increases in GFR can be seen to correspond to decreases in RVR. Acute decreases in GFR, however, correspond to acutely unchanging RVRs. The asymmetrically complementary findings hint at the haemodynamic mechanism of the acute clearance changes to be discussed.
Renal functional reserve in essential hypertension

Fig. 7. Relative change in GFR (ΔGFR/GFR = RFR), i.e. difference between test and baseline GFR before and after protein ingestion referred to baseline GFR. RFR, renal functional reserve; NT, normotensive controls (n=15); HT0, hypertensive patients during washout phase (n=16); HTt(C), hypertensive patients (chosen from HT0) after long-term antihypertensive treatment with carvedilol (n=8); HTt(F), hypertensive patients (chosen from HT0) after long-term antihypertensive treatment with fosinopril (n=8). Error bars denote SEM. The difference between NT and HT0 is highly significant (P<0.001). The difference between HT0 and HTt (C) is also highly significant (P<0.001). The difference between HT0 and HTt (F) is, however, not significant (P=0.1).

Fig. 8. RVR calculated by the formula of Gomez. NT, normotensive controls; HT0, hypertensive patients during washout phase; HTt(C), hypertensive patients (chosen from HT0) after long-term antihypertensive treatment with carvedilol; HTt(F), hypertensive patients (chosen from HT0) after long-term antihypertensive treatment with fosinopril. Difference between baseline and test RVR is significant in NT (P=0.011). Difference in baseline RVRs between NT and HT0 is highly significant (P<0.001). Difference in baseline RVRs between NT and HTt(C) is significant (P=0.0216). Difference in baseline RVRs between NT and HTt(F) is not significant.

Discussion

Using the system identification method of adapting a two-compartment model to experimental concentration profiles as illustrated in Figure 1, we found increases of GFR in normal controls, but 'paradoxical' decreases of GFR by ~10% in patients with essential hypertension by (Figure 3). These acute decreases in GFR following protein ingestion cannot be explained by systemic blood pressure effects, since there were no acute systemic changes in the MAP (Figure 2). Neither can these decreases in GFR be explained by acute mesangial contraction, since there were no concomitant decreases in ERPF (Figure 4). Instead, the dynamic renal responses to protein ingestion can be understood in terms of a preferentially pre-glomerular vascular dysfunction in patients with essential hypertension.

This situation resembles that found in the so-called captopril test where vasodilation of the efferent arterioles is achieved by the administration of captopril in kidneys with stenosed arteries, resulting in acutely decreased GFR and filtration fraction [18]. Similarly, consideration of the filtration process in renal vessel resistances differentially altered between the vasa
afferentia and the vasa efferentia should allow expectation of not only an absence of increases in GFR after protein ingestion, but even ‘paradoxical’ decreases in GFR concomitant with ERPFs that remain the same during dynamic tests. The interplay of the resistances of the vasa efferentia when remaining high and of the vasa afferentia when acutely reduced by a vasodilatory stimulus leads to an acutely reduced GFR during preservation of the renal blood flow as observed in previous studies in diabetics [19]. Since protein ingestion did not cause any acute reduction in systemic blood pressure (Figure 2), it probably interferes with glomerular filtration by reducing efferent vascular resistance in kidneys with no significant change in reactivity of the afferent vascular resistance to the dietary signal.

Impaired reactivity of afferent vascular resistance in patients suffering from long-term essential hypertension, as seen in Figures 4 and 8, has been ascribed to decreased bioavailability of nitric oxide (NO) due to increased levels of reactive oxygen species [20]. Since L-arginine has been shown to have free-radical scavenging properties [21] and to serve as a substrate for NO synthesis, it may increase the bioavailability of NO [22]; therefore, the dietary signal could be the L-arginine contained in the applied protein-rich meal.

Increased RVR in our patients, as seen in Figure 8, is explained by the established modulation of the impact of NO on renal haemodynamics and glomerular function through oxidative stress on vascular endothelial cells [23] in essential hypertension. In our patients treated with the antihypertensive and antioxidative drug carvedilol, the change in GFR following the protein load was negative before treatment, but positive after 6 months of therapy (Figure 5). In contrast, in the patients treated with the ACE-inhibitor fosinopril, the baseline and test GFRs did not differ significantly (Figure 6).

In Figure 7 the negative value of $\Delta$GFR/GFR in the untreated hypertensive patients in contrast to the positive value of $\Delta$GFR/GFR in the normotensive controls indicates a pathological alteration in renal vascular reactivity. Again, a nearly complete normalization of the haemodynamic mechanism is shown in the carvedilol-treated group, but only a partial one in the fosinopril-treated group. The high RFR in our carvedilol-treated patients, and the increased resistance to low density lipoprotein (LDL) oxidation found previously in an identical group of patients with essential hypertension [24] also treated with carvedilol show parallel long-term developments. The low RFR in our fosinopril-treated group is paralleled by the resistance to LDL oxidation remaining low in a previously studied ACE inhibitor-treated group of patients with essential hypertension [24].

Since both groups of hypertensive patients showed similar long-term decreases in MAP, and the MAPs of the two groups did not differ from each other significantly either before or after the treatments, the above effect of carvedilol on renal haemodynamics does not seem to be primarily due to reduction of blood pressure but rather to the antioxidative effect of carvedilol. Other antioxidative agents must be tested to clarify whether carvedilol plays a role in pharmacologically-mediated restoration of functional reactivity diminished by the effects of free radicals on endothelial cells [25], and whether this repair is reflected in the almost completely restored normal protein-induced acute relative change in GFR seen in our patients treated with carvedilol.

There is a significant acute decrease in RVR upon protein ingestion in the normotensive controls, whereas in the hypertensive patients during the washout period there is no acute change in RVR upon protein ingestion (Figure 8). However, while a tendency towards reconstitution of reactivity to protein stimulation was found in patients treated with carvedilol, in patients treated with fosinopril a tendency towards long-term lowering of RVR was seen. This is in accordance with the finding that fosinopril has been shown to reduce structural, but not functional alterations in small arteries by interfering with growth factors in spontaneously hypertensive rats [26].

The RVR determinations both supplement and corroborate the conclusions drawn from the determinations of renal functional reserves. Thus the concept of decreased afferent reactivity and increased afferent resistance in the hypertensive patients is supported by the increased and unchanging RVR seen during the washout period. Carvedilol appears to restore afferent reactivity, while fosinopril appears to reduce afferent resistance.

The hypertensive patients studied had normal serum creatinine concentrations, normal creatinine clearance and no proteinuria. These patients revealed baseline GFRs in the normal range over the entire treatment period. Obviously, single GFR determinations cannot detect early alterations in renal function and renal haemodynamics. These become visible only by evaluating the acute changes in GFR following a protein load.

In contrast to previous studies finding only ‘blunted increases’, our study method was sensitive enough to detect acute decreases in GFR. However, the unchanging RVR is complementary to the negative RFR in hypertensive patients as demonstrated in Figures 7 and 8. Our finding is in exact correspondence with a relevant previous finding of decreasing RVR in normotensive subjects and of unchanging RVR in patients with essential hypertension [20]. Obviously, in determining PAH clearances, which are up to five times greater than inulin or sinistrin clearances, traditional constant infusion methods will attain qualitatively the same results as modern methods of system identification within the short time horizons needed for dynamic experiments.

After a period of scepticism about the usefulness of dynamic renal function tests [27], our study demonstrates that modern compartmental analysis of kinetic clearance experiments enables detection of early stages of intrarenal haemodynamic alterations in the development of hypertension when there are pronounced disparities between pre- and post-glomerular vascular...
resistances, especially when hyperfiltration due to increased blood pressure may be present suggesting a normal GFR despite a decreased RFR and increased RVR.

The pharmacokinetic system identification method underlying the method of dynamic renal function testing enables the determination of the clearance estimates and the derived RFR and RVR values not only with the required precision, but also with the required error bounds for the system parameter estimates in individual patients [16,17]. Because of this, the method presented here allows the assessment of RFRs and RVRs to monitor the acute renal vascular effects of vasoactive agents in vivo. The method should be useful in monitoring treatment of pathophysiological changes in early stages of alterations in renal vascular properties.

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