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Systemic and intratubular effects of cyclosporin-A and tacrolimus on the rat kidney

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Abstract

Cyclosporin-A and tacrolimus can cause hypertension and renal failure through endothelin receptors. The importance of tubular function was never investigated. The aim of this study was to compare the effects of intratubular injection of cyclosporin-A and tacrolimus with effects observed during systemic infusion. In 20 rats, either cyclosporin-A or tacrolimus was infused, 30 and 1 mg/kg i.v., respectively, in 30 min. Before and after administration, glomerular filtration rate, single nephron filtration rate, proximal and distal absolute reabsorption and percent reabsorption were measured by clearance and micropuncture techniques. In 22 other rats, single nephron filtration rate, absolute reabsorption, percent reabsorption, were measured at the last proximal and early distal tubules before and during intraluminal microinjection of either cyclosporin-A or tacrolimus. During cyclosporin-A and tacrolimus i.v. infusion, glomerular filtration rate fell from 536 ± 43 to 448 ± 37 μl/min ($P < 0.026$) and from 408 ± 33 to 284 ± 81 μl/min ($P < 0.02$), single nephron filtration rate from 26.4 ± 2.0 to 20.6 ± 1.9 ($P < 0.002$) and from 21.6 ± 2.2 to 17.4 ± 2.0 nl/min, respectively ($P < 0.02$). The last proximal absolute reabsorption remained unchanged with cyclosporin-A (16.8 ± 2.2 vs. 15.1 ± 1.7 nl/min, $P > 0.444$), but was slightly reduced by tacrolimus (14.4 ± 1.7 vs. 11.3 ± 1.7 nl/min, $P < 0.05$). During microinjection, single nephron filtration rate was increased by cyclosporin-A (20 ± 1 vs. 63 ± 8 nl/min, $P < 0.0001$), and tacrolimus (from 17 ± 2 to 49 ± 9 nl/min, $P < 0.0001$), and so was reabsorption, independent of the sampling site. Cyclosporin-A and tacrolimus, indeed, raise single nephron filtration rate directly when injected intraluminally. Since this effect occurs in the direction opposite to that recorded during systemic infusion, it must be mediated through different pathways. The i.v. infusion of cyclosporin-A, but not tacrolimus, impairs glomerulo-tubular balance.

Keywords: Cyclosporine; Tacrolimus; Glomerulo-tubular balance; Tubulo-glomerular feed-back; Tubular reabsorption; Single nephron filtration rate

1. Introduction

Cyclosporin-A has become the mainstay of treatment of transplant rejection and of several autoimmune diseases. Unfortunately, it can cause adverse events, mainly hypertension and renal failure. Recent studies showed that the mechanisms leading to hypertension may be different from those causing renal failure (Textor et al., 1993). Cyclosporin-A is capable of inducing significant vasoconstriction, which seems to be mediated by endothelin ET_A receptors present in renal vessels, mainly the larger preglomerular arteries (Cavarape et al., 1998). Systemic hypertension could stem from activation of both endothelin ET_A and endothelin ET_B receptors, from the stimulation of other vasoconstricting systems, like adrenergic outflow (Murray et al., 1985), thromboxanes (Perico et al., 1992), as well as the inhibition of vasodilating antagonists (Stephan et al., 1995).

Tacrolimus, a new drug similar to cyclosporin-A, is given to patients who develop side effects from cyclosporin-A (Pham et al., 1996).

While the vascular effects of these agents have been the target of a number of studies, their selective influence on tubular reabsorption and on the macula densa has not. The macula densa could mediate some of the vascular and hypertensive effects of these substances, while tubular reabsorption is affected by endothelin, which is released by proximal tubular cells when exposed to cyclosporin-A (Haug et al., 1998).

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The present paper work examined the tubular effects of cyclosporin-A and tacrolimus during systemic infusion sufficient to generate acute hypertension, and during injection into the tubular lumen, which is entirely devoid of systemic and whole kidney effects. This design is therefore suitable to measure changes principally linked to the tubular and/or macula densa-mediated events caused by these immune suppressive agents, either directly or through endothelin release.

2. Materials and methods

The experiments were performed on 42 male rats, weighing 210 to 260 g. The rats were of a Wistar–Munich strain with a large number of superficial glomeruli, obtained by courtesy of Prof. Hartmut Osswald, Department of Pharmacology, University of Tubingen, Germany. The animals were prepared for micropuncture as previously described in detail (Romano et al., 1995). They were anesthetized with thiopental sodium, 50 mg/kg i.v., placed on a heated surgical table, and their left kidney was exposed in a lucite cup and prepared for micropuncture. The renal surface was illuminated by a cold light delivered by a fiber optic apparatus, and continuously bathed with mineral oil warmed at 37°C. The left ureter was cannulated with a polyethylene tube for the necessary urinary measurements. From both ureters, urine was returned to the animal through a urine reinfusion apparatus, already described in detail (Bartoli et al., 1996a) to maintain a constant level of drugs and glomerular markers. During the experiments, the animals were infused with a priming solution (NaCl 153 mmol/l, 20 ml/kg) followed by a maintenance solution (NaCl 153 mmol/l) at a rate of 0.26 ml/kg/min.

On each animal, we started by mapping a number of nephrons, that were identified and drawn by hand on paper after injection of one or more boluses of 2.5% Lissamine Green-stained Ringer solution, injected through a very thin-tipped pipette (< 5 μm outer diameter). The injection was performed either at the first proximal loop leaving Bowman's space, when clearly visible, or directly into Bowman's space. The progression of the dye allowed mapping of proximal and distal tubules of the same nephrons.

The experiments were performed under a double-headed Zeiss microscope, with two trained operators working simultaneously with Leitz micromanipulators.

The experiments were done under general anesthesia and in compliance with the laws regulating work on animals. More detailed descriptions of the techniques have been published elsewhere (Bartoli et al., 1996b).

We performed two different types of experiments, one with systemic infusion with either cyclosporin-A or tacrolimus, the other with microinjection inside the tubular lumen of either drug.

2.1. Protocol I: systemic injection of the test drugs

The experimental protocol required the execution of clearance and micropuncture experiments before and after the intravenous infusion of cyclosporin-A, 30 mg/kg, or tacrolimus, 1 mg/kg. The drugs were delivered i.v. over 30 min.

(1) The control samples were collected before i.v. infusion of the drugs. There were at least two clearance periods, with timed urine collection into calibrated glass capillaries and blood sampling before and after urine collection. In each animal, micropuncture collections were performed in one nephron before the first clearance period, in another nephron after the last clearance period, and between clearance periods in two additional nephrons.

The samples were obtained with the total collection technique, either at the early distal or the last proximal sampling site of different nephrons. This control phase of the experiment lasted 87 ± 3 min.

(2) The experimental samples were collected after the i.v. infusion of the drugs. Again, at least two clearance periods were performed, with the same modalities described above. Micropuncture recollections were taken from the same sites of the same nephrons sampled during control conditions. This experimental phase lasted 123 ± 4 min.

Blood pressure was measured with a strain gauge (2-Channel Recorder ''Gemini 2'', Basile Biological Research apparatus, Comerio, Varese, Italy) via the femoral artery catheter that was also used for blood sampling.

2.2. Protocol II: tubular lumen microinjection experiments

The experimental protocol required three samplings from the same nephrons.

(1) Baseline free-flow total collection either at the early distal tubule, or at the last proximal convolution of the proximal tubule accessible to micropuncture on the renal surface. This collection was performed by injecting black-stained oil into the tubular lumen. When the oil column, approximately eight tubular diameters long, had flowed past the pipette tip, tubular fluid sampling was started by aspirating all the tubular fluid reaching the tip of the collecting pipette, during oil blockade of the tubular segment immediately downstream from the site of sampling. The technique of total collection of tubular fluid has been described previously (Romano et al., 1998).

(2) Microinjection of test substances into the tubular lumen. After the baseline collection, a thin-tipped microinjecting pipette was inserted into the tubular lumen. The puncturing site for microinjecting test substances was:

(a) The last proximal segment when the baseline collection had been performed at the early distal segment. This occurred in 27 instances.

(b) The early proximal or Bowman's space when the

baseline collection had been performed at the last proximal segment. This occurred in 55 instances.

Microinjection was performed manually, by continuous steady injection of Lissamine Green-stained fluid. When the green dye had reached a stable intensity in the nephron, we performed a free flow total collection from the same site that was sampled during the baseline collection, with the same technique. If the oil block of the baseline collection had remained in situ, the tubule was decompressed by aspirating the oil column through a different thin-tipped pipette. If the tubule had been damaged by the previous punctures, the collection was performed upstream in the same superficial convolution, by sealing the previous puncture sites with the distal oil block.

(3) Post-control collection. After microinjection had been interrupted, the tubule was decompressed (see above). Then, after an interval of at least 10 transit times, and when the green dye had completely disappeared, the total collection was repeated either from the same site or slightly upstream.

The microinjection fluid had the following composition: NaCl 153 mmol/l, Lissamine Green 1%, [^{14}C]carboxy-inulin 11 mCi/l. The test substances were:

(A) Cyclosporin-A, 20 μg/ml, in 33 proximal and 11 distal microinjections;
(B) Tacrolimus, 1 μg/ml, in 22 proximal and 16 distal microinjections.

These concentrations were selected to reach luminal values of either drug in the range of those calculated for the i.v. infusion experiments. The i.v. doses selected were twice the full acute immunosuppressive amounts, and were those consistently used in murine experiments to reproduce the acute onset of hypertension and glomerular filtration rate deterioration (Cavarape et al., 1998; Sabbatini et al., 1989).

The injectate was prepared by diluting an i.v. preparation of cyclosporin-A (Sandimmun 50 mg/ml) or tacrolimus (Prograf 10 mg/ml) with suitable amounts of saline. The vehicle was the same for tacrolimus and cyclosporin-A, and its final concentration was 40 times less than that in the commercial i.v. preparation. No effect of vehicle alone had been found in previous experiments (Sabbatini et al., 1989; Hadad et al., 1995).

In each collected sample, we measured the tubular fluid volume in nanoliters (nl), and the time of collection in minutes (min). We also measured the counts per minute (cpm) of [^{3}H]methoxy-inulin infused systemically, and the cpm of carboxy-inulin added to the microinjection fluid.

Counting was performed with a Tri-carb instrument (B1900TR, Tricarb Liquid Scintillation Analyzer, Packard-Canberra Company, Groningen, The Netherlands) with a program that allows separate computation of the tritiated and [^{14}C] markers. For further details, see previous publication (Romano et al., 1997). The [^{3}H]methoxy-inulin was also measured in plasma samples drawn immediately before or after the puncturing of each nephron.

For each collected sample, we calculated the following.

(I) The tubular fluid-to-plasma (and/or to injected fluid) inulin concentration ratio (TF/P);

(II) The fractional reabsorption (1 − P/TF);

(III) The collection rate of tubular fluid in nl/min (CR);

(IV) The nephron filtration rate (SNGFR) given by CR × TF/P, expressed in nl/min. While the values measured with tritiated inulin were used for calculating the SNGFR, those measured with carboxy-inulin were used to calculate the average microinjection rate in nl/min;

(V) The absolute rate of reabsorption (AR), in nl/min, given by SNGFR-CR; The values obtained during microinjection had to be corrected for the dilution by the injected fluid with the formulas already published for previous microinjection studies (Romano et al., 1997).

The whole kidney clearance calculations for glomerular filtration rate were based on the [^{3}H]methoxy-inulin concentrations measured simultaneously in urine and plasma. Na^+ and K^+ were also measured in each urine sample and in a blood sample obtained by exsanguination of the animal at the end of the experiment.

Cyclosporin-A and tacrolimus were measured in plasma 2 h after the end of the i.v. infusion with the ABBOTT monoclonal assays (Moyer et al., 1991; Jusko et al., 1995).

Table 1
Systemic and micropuncture data following i.v. infusion of cyclosporin-A and tacrolimus

	Cyclosporin-A				Tacrolimus			
	Baseline	i.v. infusion	*P*	*N*	Baseline	i.v. infusion	*P*	*N*
Mean BP (mmHg)	102 ± 2	111 ± 3	0.0001	14	96 ± 1	105 ± 1	0.0001	6
GFR (μl/min)	536 ± 43	448 ± 37	0.026	14	408 ± 33	284 ± 81	0.02	6
V/GFR%	3.5 ± 0.5	6.1 ± 0.7	0.004	14	2.3 ± 0.2	5.0 ± 0.1	0.0001	6
SNGFR (nl/min)	26.4 ± 2.0	20.6 ± 1.9	0.002	14	21.6 ± 2.2	17.4 ± 2.0	0.02	6

The table shows the effect of systemic infusion of cyclosporin-A and tacrolimus on mean blood pressure (BP in millimeters of mercury), glomerular filtration rate (GFR) from the left (experimental) kidney in microliters per minute, the percentage ratio between urine flow rate and glomerular filtration rate (V/GFR%), and the rate of filtration of single nephrons (SNGFR in nanoliters per minute) are also reported as means and standard error of the mean. *N* is the number of animals, *P* the statistical probability by paired *t*-test.

The data were analyzed statistically. Means and standard errors of the mean were computed and their differences tested with paired or unpaired *t*-tests. The statistical program, ''Stat Work'', with a Macintosh LC II personal computer was used.

3. Results

This study included clearance and micropuncture data obtained during systemic drug injection (Protocol I) and micropuncture data measured during intratubular drug injection (Protocol II).

The infusion of 30 mg/kg i.v. of cyclosporin-A data are reported in Table 1. Mean blood pressure rose from 102 ± 2 to 111 ± 3 mmHg ($P < 0.0001$), while glomerular filtration rate of the left kidney fell by 16.4%. The ratio between urine flow rate and glomerular filtration rate (V/GFR%) rose from 3.5 ± 0.5 to $6.0 \pm 0.7\%$ ($P < 0.004$). Concomitant with these whole kidney measurements, the nephron filtration rate fell from 26.4 ± 2.0 to 20.6 ± 1.9 nl/min ($P < 0.002$).

The data obtained before and after the i.v. infusion of tacrolimus 1 mg/kg is similar to those obtained with cyclosporin-A.

Table 2 gives the results obtained for samples from the early distal and the last proximal tubules of different nephrons during intravenous infusion of the two drugs. The single nephron filtration rate fell both with cyclosporin-A and tacrolimus. Reabsorption was unchanged with cyclosporin-A. During tacrolimus administration, instead, when measured both at the early distal and last proximal sampling site, absolute resorption decreased. This indicates disruption of glomerulo-tubular balance by cyclosporin-A only.

Protocol II consisted of micropuncture measurements, before, during and after microinjection of either drug into the tubular lumen.

Table 3 gives the results obtained with microinjection of cyclosporin-A.

When the drug was injected into the early proximal segment, or Bowman's space, single nephron filtration rate and absolute reabsorption rose significantly, while the rise in percentage reabsorption was more limited and non-significant. When the microinjection was performed at the last proximal segment and collection was from the early distal segment, similar results were obtained.

Table 3
Micropuncture data during microinjection of cyclosporin-A

	Baseline	CyA	*P*	*N*	[CyA] ng/ml
SNGFR LP (nl/min)	19.9 ± 1.4	56.3 ± 8.6	0.0001	33	4838 ± 681
PR LP%	59 ± 4	65 ± 7	0.338	33	
AR LP (nl/min)	12.4 ± 1.3	43.8 ± 8.3	0.0001	33	
SNGFR ED (nl/min)	19.0 ± 3.1	69.2 ± 15.3	0.006	11	6088 ± 934
PR ED%	78 ± 4	90 ± 5	0.06	11	
AR ED (nl/min)	15.4 ± 2.9	65.6 ± 15.3	0.005	11	

The paired microinjection data obtained during cyclosporin-A(CyA) microinjection are reported separately for samples collected from last proximal and early distal tubular segments. The concentration of the drug in the tubular fluid is reported in nanograms per millilitre. For the meaning of the other symbols, see Tables 1 and 2.

The rate of microinjection averaged 3.7 ± 0.5 nl/min, which is $6.7 \pm 1.3\%$ of the single nephron filtration rate. Therefore, the dilution of filtered substances other than NaCl was negligible.

The concentration of cyclosporin-A into the tubular lumen could be calculated from that present in the injectate and from the microinjection rate, single nephron filtration rate and fractional reabsorption. It averaged 4838 ± 681 in the last proximal, and 6088 ± 934 ng/ml in the early distal. The value measured in plasma after i.v. infusion (3033 ± 77 ng/ml) yielded similar tubular fluid concentrations after correction for tubular reabsorption.

Table 4 reports the results obtained before and during microinjection of tacrolimus into the tubular lumen. Single nephron filtration rate and absolute reabsorption rose significantly for both proximal and distal perfusion with the drug. Although percentage reabsorption did not change significantly, it actually decreased in distal collections. In agreement with these micropuncture results, the percentage of filtered volume, which was excreted (V/GFR%, Table 1) was significantly higher compared to the baseline level

Table 2
Paired (distal or proximal basal vs. experimental) data during i.v. infusion of cyclosporin-A and tacrolimus

	Cyclosporin-A				Tacrolimus			
	Baseline	i.v. infusion	*P*	*N*	Baseline	i.v. infusion	*P*	*N*
SNGFR LP (nl/min)	27.8 ± 2.3	22.7 ± 2.2	0.016	42	22.1 ± 1.7	17.3 ± 1.5	0.002	13
PR LP%	56 ± 3	66 ± 4	0.053	42	64 ± 6	63 ± 6	0.875	13
AR LP (nl/min)	16.8 ± 2.2	15.1 ± 1.7	0.444	42	14.4 ± 1.7	11.3 ± 1.7	0.050	13
SNGFR ED (nl/min)	21.9 ± 4.4	13.9 ± 3.4	0.040	13	19.9 ± 2.6	13.4 ± 2.3	0.040	14
PR ED%	57 ± 8	74 ± 7	0.111	13	75 ± 5	41 ± 11	0.025	14
AR ED (nl/min)	13.3 ± 3.5	11.7 ± 3.1	0.612	13	15.2 ± 2.5	4.6 ± 1.7	0.001	14

The table shows the effect of systemic infusion of cyclosporin-A and tacrolimus on SNGFR, percentage reasorption (PR), and absolute reasorption (AR). The data obtained with collections from the last proximal(LP) sampling site are reported separately from those obtained in samples taken at the early distal tubule (ED). *N* is the number of paired measurements.

Table 4
Micropuncture data during microinjection of tacrolimus

	Baseline	Tc	*P*	*N*	[Tc] ng/ml
SNGFR LP (nl/min)	16.0 ± 1.9	45.9 ± 13.0	0.02	22	568 ± 65
PR LP%	42 ± 8	49 ± 8	0.49	22	
AR LP (nl/min)	7.5 ± 1.7	31.9 ± 8.9	0.025	22	
SNGFR ED (nl/min)	17.4 ± 2.5	53.0 ± 11.0	0.01	16	547 ± 45
PR ED%	74 ± 6	64 ± 9	0.25	16	
AR ED (nl/min)	12.1 ± 2.0	39.5 ± 12.0	0.04	16	

The paired microinjection data obtained during tacrolimus (Tc) microinjection are reported separately for samples collected from last proximal and early distal tubular segments. The concentration of the drug in the tubular fluid is reported in nanograms per millilitre. For the meaning of the other symbols, see previous tables.

than was that measured with cyclosporin-A (217 vs. 174%, $P < 0.05$).

4. Discussion

Cyclosporin-A causes hypertension, due to an agonist action on systemic endothelin ET_A receptors, and, through independent mechanisms (Textor et al., 1993), renal failure, which could be attributed to exclusively intrarenal, presumably tubular events. It has been reported that cyclosporin-A induces the release of endothelin by tubular cells (Haug et al., 1998). In turn, we showed that endothelin, injected into the tubular lumen of rat nephrons, evokes a rise in single nephron filtration rate and absolute reabsorption (Romano et al, 2000b). Based on this, we compared the systemic effects of i.v. administration of cyclosporin-A with effects measured during intra-tubular microinjection of the substance, in an attempt to separate events leading to hypertension from effects responsible for renal failure. As a related issue, we used both cyclosporin-A and tacrolimus to verify whether the purported advantage of the latter agent is linked to some intrarenal and tubular effect associated with the onset of renal failure.

The present results for systemic infusion of hypertensive doses of cyclosporin-A confirmed that the agent depresses glomerular filtration rate and single nephron filtration rate proportionately, mimicking the effects of endothelin infusion.

The critical experiment performed with microinjection of the substance yielded opposite results: single nephron filtration rate, absolute and fractional reabsorptions rose together. It is noteworthy that these results were independent of the sampling site, whether this was the last convolution of the proximal tubule or the earliest distal segment accessible to micropuncture on the renal surface. The data obtained from microinjection duplicate those obtained during microinjection of endothelin into the tubular lumen (Romano et al, 2000b).

Therefore, the present data, together with previous results from our laboratory and the literature, suggest that cyclosporin-A acts through endothelin release and/or endothelin receptors. However, while the response to systemic infusion could be attributed to an agonist action on endothelin ET_A receptors, a preferential activation of endothelin ET_B receptors is difficult to reconcile with results of the microinjection studies, since the substance was injected into the lumen, while the receptors are vascular.

Thus, a more complex explanation is required to account for the unexpected vasodilating properties of this powerful vasoconstrictor when put directly in contact with the luminal side of proximal tubular cells.

We are tempted to speculate that powerfully vasoconstricting peptides, while stimulating their vessel receptors, are simultaneously filtered by the glomerulus and released into the tubular lumen. They might then act intraluminally, modulating Na and water transepithelial fluxes, as well as triggering responses that can release vasodilators from the peritubular side of the tubule. The vasodilating substances could then counterbalance the direct vasoconstriction, generating a close match between competing influences, resulting in stability of renal hemodynamics and glomerular filtration. In fact, angiotensin causes intrarenal vasoconstriction and modulates proximal reabsorption (Quan and Baum, 1999), endothelin and secretin cause vasoconstriction while they increase both single nephron filtration rate and reabsorption when injected into the lumen (Romano et al., 2000a). In contrast, non-peptidergic constrictors evoke the vascular responses while they do not up-regulate single nephron filtration rate during microinjection (Romano et al., 1999). It then seems that there could be a generalized system whereby peptidergic vasoconstrictors can trigger a vasodilator response when they reach the tubular lumen, avoiding abrupt drops in filtration and the sudden onset of renal failure.

However, were this an in-built system aimed at allowing the full expression of a homeostatic vasoconstricting response while dampening and smoothing its effects on salt and water excretion, it should protect from acute renal failure, while cyclosporin-A does in fact cause it. We believe that it is possible to reconcile this apparent contradiction.

The tables show that the microinjection of cyclosporin-A disrupts the phenomenon of glomerulo-tubular balance: in fact, when the single nephron filtration rate rises, absolute reabsorption rises even more with microinjection. During i.v. infusion, single nephron filtration rate falls, while absolute reabsorption does not change significantly. While glomerulo-tubular balance produces a close adaptation of the changes in reabsorption to those in filtration (Bartoli, 1981), its absence leads to wide fluctuations in delivery from the proximal tubule in response to changes in single nephron filtration rate. In the present experiments, the lack of glomerulo-tubular balance during i.v. infusion led to an exaggerated fall in fluid delivery, reflected by a fell of the measured collection rate to 7.2 ± 0.8 nl/min. This change itself, through the tubulo-glomerular feedback system,

should up-regulate single nephron filtration rate and restore stability (Häberle and Davis, 1984, Schnermann et al., 1998). However, tubulo-glomerular feedback responds to reductions in delivery up to 10 nl/min, while it becomes unresponsive below this value (Wright et al., 1982). In consequence, cyclosporin-A protects the kidney from acute renal failure through the release of endothelin, but at the same time conveys the risk of acute renal failure by disrupting the glomerulo-tubular balance: when the delivery falls below a threshold value in the neighbourhood of 10 nl/min, tubulo-glomerular feedback cannot up-regulate single nephron filtration rate and a further fall in filtration ends in an abrupt drop in delivery and progression towards renal failure. It is important to notice that tacrolimus does not disrupt the glomerulo-tubular balance, and does not increase reabsorption during microinjection to the same extent as doe cyclosporin-A. This apparently slight difference in the effects of these two quite similar drugs could account for the lower chance of developing acute renal failure during tacrolimus administration. The better preservation of glomerulo-tubular balance by tacrolimus could be the clue to its lesser renal toxicity.

In summary, cyclosporin-A, presumably through endothelin receptors and release, exerts a dual action: on one hand, it causes systemic and renal vasoconstriction, and a fall in glomerular filtration rate. On the other hand, from the tubular lumen, it up-regulates the single nephron filtration rate. During systemic infusion of hypertensive amounts of the drug, the vasoconstricting effect prevails, such that filtration falls to a limited extent. During intratubular injection, in the absence of stimulation of vascular endothelin ET_A receptors, only the unopposed up-regulation of glomerular filtration rate will be measured, and this was seen in our experiments.

We also designed our study to compare data obtained when the macula densa was exposed to the drug with data obtained during perfusion of the proximal tubule only. In the former experiments, the injections were performed into the late proximal tubule while the tubular fluid was collected from the distal tubule, past the macula densa segment. In the latter experiments, the macula densa was excluded by the oil blockade inserted to collect fluid from the late proximal tubule, while microinjection was being performed from Bowman's space. The up-regulation of single nephron filtration rate was almost identical under these different circumstances. This does not allow us to state that the up-regulation of glomerular filtration rate is mediated through the macula densa, although the drugs, which are lipophylic and diffusable (Venkataramanan et al., 1991), could reach their vascular targets and/or the macula densa even during proximal injection. As an alternative explanation, we should mention the fact that endothelin, released by the proximal cells by cyclosporin-A, could trigger the release of the vasodilating nitric oxide from the peritubular vascular endothelium (Hirata et al., 1995). The macula densa effect, if present, could be mediated by the inhibition of the renin–angiotensin system by cyclosporin-A (Textor et al., 1993), resulting in preferential dilatation of the efferent resistor, and an attendant fall in filtration pressure (Hricik et al, 1983).

In conclusion, the data obtained with these experiments demonstrate that cyclosporin-A blunts the phenomenon of glomerulo-tubular balance in the proximal tubule of the rat kidney. In the presence of drug-induced renal vasoconstriction, the fall in glomerular filtration rate is accompanied by an exaggerated drop in tubular fluid delivery to the macula densa, which abolishes the tubulo-glomerular feedback response and makes glomerular filtration rate unstable, leading to the possible onset of sudden, unopposed vasoconstriction and renal failure. The drugs are capable of resetting and magnifying the sensitivity threshold of the macula densa sensor, partially protecting the kidney from the effects of vasoconstriction. However, the systemic effects and the drug-dependent direct vascular reactivity can overwhelm the tubulo-glomerular feedback and precipitate overt renal failure.

Tacrolimus can cause a marked inhibition of proximal reabsorption, leading to higher macula densa delivery and urine flow rate. This could represent an additional protective mechanism with respect to that afforded by cyclosporin-A.

This final point emphasizes the advantage of maintaining a reasonable degree of volume expansion, accompanied by inhibition of proximal reabsorption and a consequent large proximal delivery and urine flow rate, as a simple and effective way to prevent cyclosporin-induced renal failure.

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