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Cardiovascular 24-h rhythms and renal excretory function in rats after allogeneic kidney transplantation

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Abstract We investigated long-term and circadian blood-pressure changes in a Fischer → Lewis rat model of chronic renal allograft rejection. Ten days after allogeneic kidney transplantation, rats were equipped with telemetry transmitters for continuous monitoring of blood pressure. Urine was sampled 24, 96 and 180 days after kidney transplantation for measurement of renal protein loss and excretion of nitric oxide metabolites. Proteinuria increased with time after transplantation, while urinary excretion of nitric oxide metabolites declined. In five of six rats blood pressure remained in the normotensive range, and its 24-h pattern was preserved up to 6 months post-transplantation. One animal showed hypertensive blood pressure and a disturbed 24-h pattern with peak values during the rest period. In the post-mortem analysis this rat showed a hydronephrotic kidney graft. In conclusion, blood pressure and its 24-h pattern are preserved after successful allogeneic kidney transplantation in the rat.

Keywords Allograft rejection · Blood pressure · Radiotelemetry · Circadian rhythm · Rat

Introduction

Hypertension is a frequent finding after kidney transplantation, with a prevalence of 50–90% in post-transplant patients [15, 28]. Impaired renal function as a consequence of chronic allograft rejection and cyclosporine therapy are thought to represent the major factors contributing to the development of hypertension after kidney transplantation. In addition to the general increase in blood pressure, these patients often show a blunted nocturnal blood-pressure fall [4, 19, 20, 25]. The prevalence of this 'non-dipping' blood-pressure profile was found to be 90% in the presence of chronic transplant nephropathy [16]. On the other hand, it has been shown that a blunted circadian blood-pressure pattern in patients with end-stage renal disease could be normalized with successful kidney transplantation [10]. Thus, it is not clear whether kidney transplantation itself or allograft nephropathy is mainly responsible for the blunted circadian blood-pressure variation. Moreover, immunosuppressive therapy with cyclosporine may also contribute to the loss of nocturnal blood-pressure fall after kidney transplantation [19, 25]. Studies in appropriate animal models of kidney transplantation and allograft rejection could help to identify the mechanisms involved in these long-term cardiovascular complications.

The F344 → LEW strain combination, i.e., transplantation of a Fischer 344 rat kidney into a bilaterally nephrectomized Lewis rat, has been used extensively in experimental studies of chronic renal allograft rejection [2, 3, 5, 6, 8, 9, 14, 23, 26]. In this model we demonstrated that creatinine clearance is significantly lower than in isograft recipients 2 weeks after surgery, but does not further decline for up to 24 weeks post-transplantation [6]. Histological analysis of the kidney allografts showed

mild-to-moderate transplant glomerulopathy and tubulointerstitial fibrosis 24 weeks after transplantation [6]. However, discrepant findings have been reported on long-term changes in blood pressure in this animal model, and no data are available on cardiovascular 24-h profiles. Therefore, in the present study, we performed telemetric blood-pressure monitoring in rats for up to 180 days after allogeneic kidney transplantation.

Materials and methods

Animals

Inbred male Fischer 344 rats (F344, $RT1^{lvl}$) and male Lewis rats (LEW, $RT1^{l}$), weighing between 200 and 220 g, were used as graft donors and recipients, respectively. Animals were purchased from Harlan Winkelmann, Borchen, Germany. Throughout the study, the animals were kept in alternating light–dark cycles (LD 12:12, light 7–19 h, 100 lux), at an ambient temperature of 24 ± 1 °C, with free access to water and food (standard rodent diet, Altromin, Germany; protein content 19%, Na^+ 0.72%). The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), and was approved by German federal regulations (RP Karlsruhe, Az. 35–9185.81/48/99).

Kidney transplantation

The microsurgical technique applied in this study is a modification of the technique described by Lee [17]. All surgical procedures were performed under enflurane anesthesia. The donor kidney was flushed with 1 ml of ice-cooled University of Wisconsin solution and transplanted into the recipient rat, from which the left kidney had been removed. Perfusion of the grafted organ was restored by releasing the vessel clamps after 45 min. At the end of surgery, rats were given 100,000 units of penicillin intraperitoneally. For 10 days after transplantation, the animals received cyclosporine (5 mg/kg per day intramuscularly, Sandimmun, Novartis, Germany).

Transmitter implantation

At day 10 after transplantation, the animals were anesthetized with enflurane, and the right kidney was removed. Thereafter, radiotransmitters for continuous monitoring of blood pressure, heart rate and locomotor activity (TA11PA-C40, Data Sciences International, St. Paul, Minn., USA) were implanted as described [18] with the following modifications: in order to avoid any manipulation close to the grafted kidney, we stopped blood flow in the abdominal aorta by distal compression of the vessel rather than by placing a temporary ligature. The aorta was punctured at the bifurcation with a 22-gauge needle, and the catheter was advanced cranially 6–8 mm, so that the catheter tip remained distal to the transplant artery. Cardiovascular parameters were recorded telemetrically at 5-min intervals for 6 months after implantation. Due to transmitter failure in two animals, the cardiovascular data sampled later than 3 months after kidney transplantation are from the remaining three animals. The corresponding cardiovascular data are shown graphically, but the statistical analysis was restricted to the first 94 days of monitoring. In order to prolong battery life, we switched off the remaining transmitters intermittently during the long-term follow-up period (from days 94 to 110, and 124 to 140 after transplantation).

Renal excretion

Urine sampling was carried out in standard metabolic cages (TSE, Bad Homburg, Germany) 24, 96 and 180 days after kidney transplantation. During the sampling period, animals were starved and received deionized water in order to prevent contamination with exogenous nitrate and nitrite. Urine samples were collected in 12-h intervals (0700–1900 and 1900–0700 h) and kept frozen at −20 °C until required for analysis. The urinary excretion of protein was quantified with Coomassie-Plus reagent (Pierce, Oud-Beijerland, The Netherlands), with bovine serum albumin as standard. The concentration of nitrite and nitrate (NOx) was measured colorimetrically with a commercially available assay kit (Boehringer, Mannheim, Germany) as described in detail [12]. The assay procedure is based on the enzymatic conversion of nitrate to nitrite by the nitrate reductase, followed by spectrophotometric quantification of the nitrite concentration by the Griess reagent [13]. Potassium nitrate was used as standard. Urinary electrolytes and creatinine were measured by standard laboratory methods.

Data analysis and statistics

Telemetry data were collected in 24-h segments and analyzed with DQ-Fit software [27]. Rhythm analysis was carried out in 96-h data segments early (days 27–31) and late (days 90–94) after kidney transplantation by the fitting of partial Fourier series as described in detail [27]. The mesor (24-h mean of the fitted curve), the amplitude of the 24-h rhythmic component and its acrophase (time of maximum) were used for statistical comparison. Changes in urine excretion between day and night were evaluated with the Student's *t*-test for paired data; changes with time after transplantation were tested by ANOVA for repeated measures; $P < 0.05$ was considered statistically significant.

Results

Of six rats that had undergone transplantation successfully, one developed signs of severe systemic disease and had to be killed 40 days after transplantation. The postmortem analysis revealed hydronephrosis and fluid in the abdomen, indicating dysfunction of the transplanted kidney and uremic peritoneal inflammation. This animal was excluded from the group analysis.

After the second surgical intervention, i.e., unilateral nephrectomy and transmitter implantation, full recovery of the animals required approximately 2 weeks (Fig. 1), as shown by the continuous increase in 24-h locomotor activity and by the stabilization of blood pressure. At the end of this recovery period, locomotor activity, blood pressure, and heart rate showed clear 24-h rhythmicity (Fig. 2A). Rhythm analysis in individual rats revealed significant 24-h rhythms of heart rate and activity in all animals, and of blood pressure in four of five rats (Table 1). Both the 24-h means in blood pressure and heart rate (Fig. 1) and their circadian patterns (Fig. 2) were remarkably stable until the end of the study period. The 24-h amplitudes were not different 4 and 13 weeks after kidney transplantation, but there was a minor decrease in the mesor of the heart rate, and a small shift in the 24-h acrophase of locomotor activity

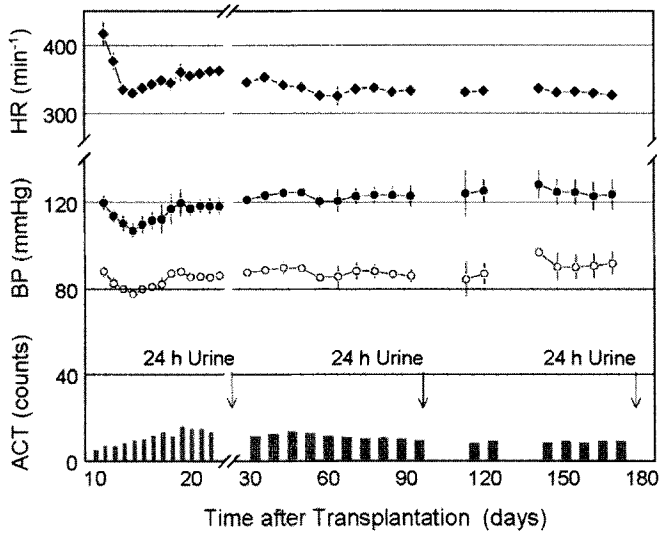

Fig. 1 Long-term changes in locomotor activity (*bars*), systolic (*closed circles*) and diastolic (*open circles*) blood pressure and heart rate (*diamonds*) in rats after allogeneic kidney transplantation. Data are shown as 24-h means for the first 2 weeks after implantation, and as weekly averages thereafter. Urine sampling was carried out early after recovery (day 24) and in the middle (day 96) and end (day 180) of the study period. Mean values ± SEM

(Table 1). At the end of the study, i.e., 6 months after transplantation, the 24-h patterns were still preserved (Fig. 2C).

Urine samples during day and night were taken early after the recovery period (day 24 after kidney transplantation), in the middle and at the end of the study (days 96 and 180, respectively). At day 24 after transplantation, all the variables studied, except sodium excretion, showed significant day–night differences: at night there was an increase in diuresis (Fig. 3A), excretion of creatinine (Fig. 3B), protein (Fig. 3C), nitric oxide metabolites (NOx, Fig. 3D), and potassium (Fig. 3E). The day–night variation was preserved in most variables at day 96, but was lost 6 months after kidney transplantation. However, the urinary excretion of potassium remained 'rhythmic' until 6 months post-transplantation, and that of sodium showed an inverse day–night variation with greater amounts excreted during the day than at night (Fig. 3F). There was a significant decrease in diuresis at night (1900–0700 h) from 24 to 180 days after transplantation (ANOVA, $P < 0.05$), while the reduction in daytime diuresis did not achieve statistical significance. Water intake closely matched the changes in 24-h diuresis (not shown). Creatinine excretion was slightly higher at day 96 than at days 24 and 180 (Fig. 3B). Urinary NOx excretion declined with time after transplantation, both during the day and night (ANOVA, $P < 0.05$ and $P < 0.01$, respectively), while proteinuria showed a continuous increase from 24 to 96 and 180 days after transplantation during the day (not significant) and night (ANOVA, $P < 0.01$). Excretion of sodium and potassium was unchanged from 24 to 180 days post-transplantation (ANOVA, not significant).

The diseased rat, excluded from the group data analysis, showed a markedly depressed locomotor activity and hypertensive blood-pressure values (Fig. 4). Most interestingly, the 24-h pattern in blood pressure was severely disturbed in this animal, with peak values occurring during daytime, while the 24-h rhythm in heart rate was largely preserved. Urinary excretion data showed that this animal excreted more urine and fewer nitric oxide metabolites than the rats that had successfully undergone transplantation, while proteinuria was not different (Fig. 4). Measurement of urine osmolality showed a loss of concentrating capability of the kidney graft (310 and 260 mosmol/kg during day and night, respectively).

Discussion

The allogeneic (F344 → LEW) kidney transplantation in rats represents an animal model of chronic renal allograft rejection, characterized by the development of progressive albuminuria, glomerulosclerosis, and a reduction in glomerular filtration and renal plasma flow [9]. The present study is the first to show that circadian blood-pressure and heart rate rhythms are preserved in this rat model. Moreover, 24-h blood pressure remained in the normotensive range throughout the 6 months follow-up, confirming a recent publication by Sanders et al. [22] who monitored blood pressure with the same radiotelemetry technique up to 8 weeks after allogeneic kidney transplantation. The absence of hypertension and the preserved 24-h blood-pressure profile in these animals was an unexpected finding, because hypertension and a non-dipping blood-pressure pattern is frequently observed after human renal transplantation [4, 19, 20, 25], and the prevalence of non-dipping blood-pressure is even higher in the presence of chronic transplant nephropathy [16]. Thus, there is a discrepancy between our experimental data and clinical observations, suggesting that elevated blood pressure and the loss of its 24-h variation cannot be attributed to kidney transplantation and graft rejection per se, but must be influenced by additional factors associated with the treatment of renal transplant recipients. Immunosuppressive treatment by cyclosporine is known to elevate blood pressure in rats [11], and is thought to contribute to the changes in the 24-h blood-pressure pattern in transplant recipients [19, 25], but the mechanisms involved are still unclear. In the present study, we gave cyclosporine for 10 days after transplantation in order to prevent acute rejection of the grafted kidney. Thereafter, we stopped immunosuppressive therapy to enable the

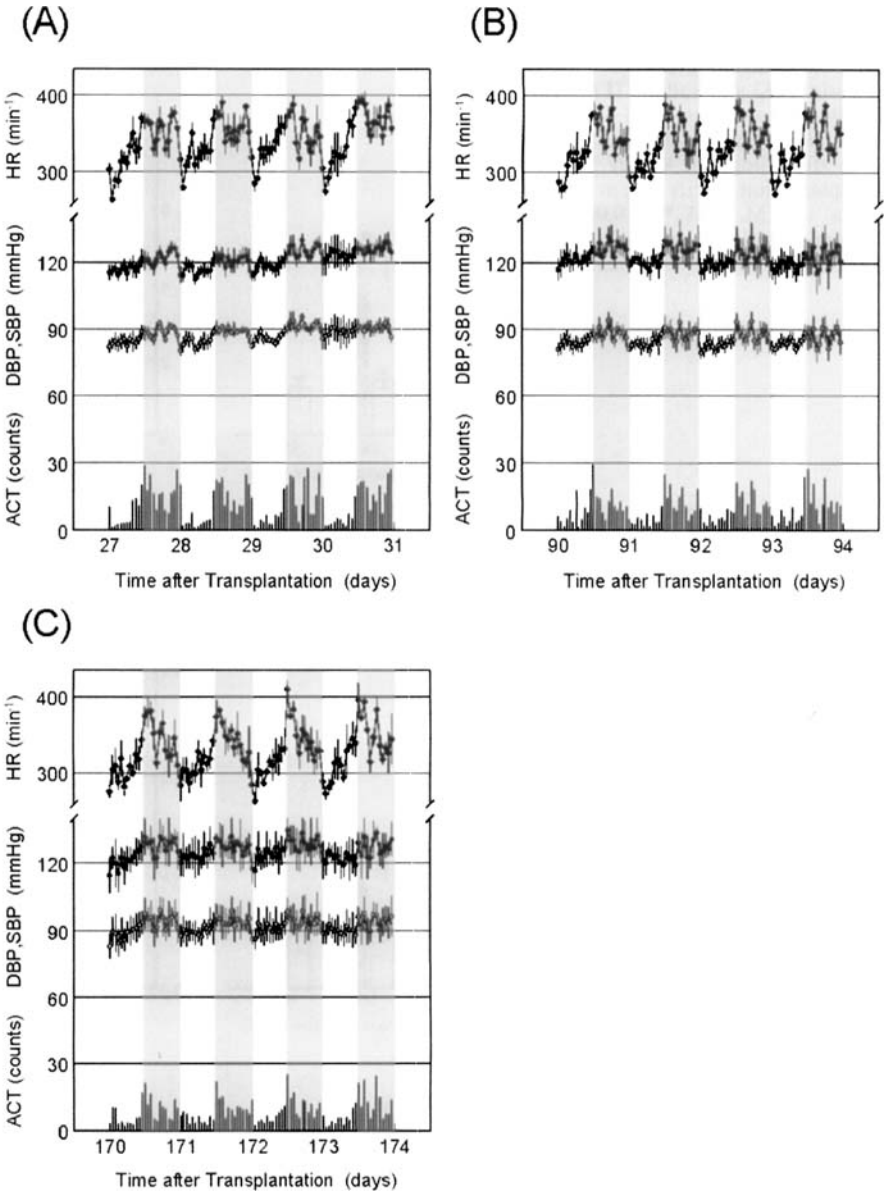

Fig. 2A–C Circadian patterns in locomotor activity (*bars*), systolic (*closed circles*), diastolic (*open circles*) blood pressure, and heart rate (*diamonds*) at different times after allogeneic kidney transplantation in rats: early after recovery (**A**), in the middle (**B**) and at the end (**C**) of the study period. *The shaded area* indicates the dark phase (1900–0700 h). Mean values ± SEM, (**A**) and (**B**) $n = 5$, (**C**) $n = 3$

Table 1 Parameters of 24-h rhythmicity in blood pressure (*BP*), heart rate and locomotor activity in rats after kidney transplantation (*KT*). Mean values ± SEM

Parameter	Time after KT (days)	Systolic BP (mm Hg)	Diastolic BP (mm Hg)	Heart Rate (permin)	Activity (counts)
Mesor	27	121.4 ± 2.4	88.1 ± 1.8	342.4 ± 2.6	5.7 ± 0.5
	90	122.8 ± 4.8	86.0 ± 2.9	334.3 ± 3.2[a]	4.8 ± 0.4
24-h-Amplitude	27	3.8 ± 0.3	3.4 ± 0.7	29.7 ± 4.5	3.5 ± 0.3
	90	4.4 ± 0.7	4.2 ± 0.5	33.1 ± 2.1	2.7 ± 0.4
24-h-Acrophase		(h:min)	(h:min)	(h:min)	(h:min)
	27	0:44 ± 0:34	0:17 ± 0:17	22:08 ± 0:35	23:56 ± 0:20
	90	3:14 ± 2:36	2:44 ± 2:41	22:20 ± 0:14	22:33 ± 0:29[a]
		$n =$	$n =$	$n =$	$n =$
Significant rhythm	27	4	4	5	5
	90	5	5	5	5

[a] $P < 0.05$, Student's *t*-test, 27 versus 90 days after kidney transplantation

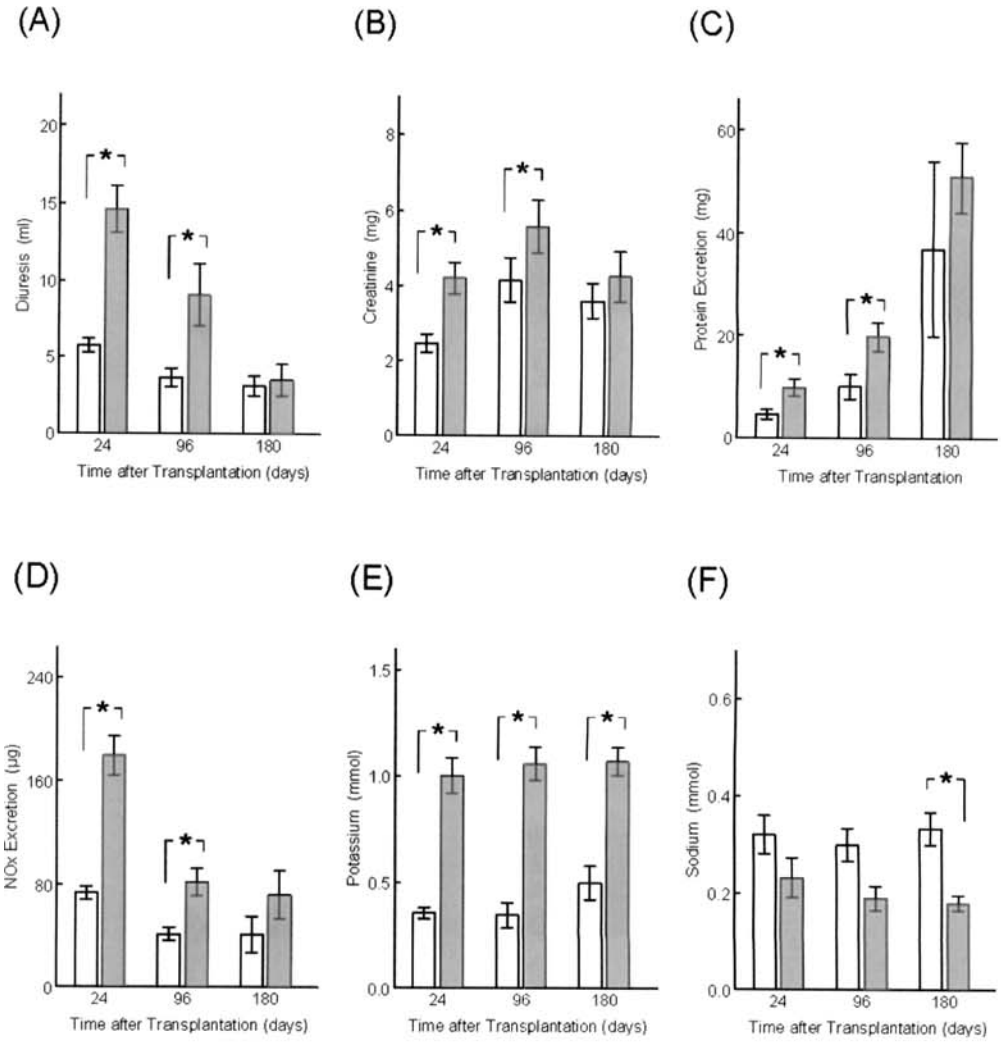

Fig. 3 Diuresis (**A**), excretion of creatinine (**B**), protein (**C**), nitric oxide metabolites (**D**), potassium (**E**) and sodium (**F**), during day (*open bars*) and night (*closed bars*) at different times after allogeneic kidney transplantation in rats. Mean values ± SEM, $n = 5$, $^*P < 0.05$, Student's *t*-test, day versus night

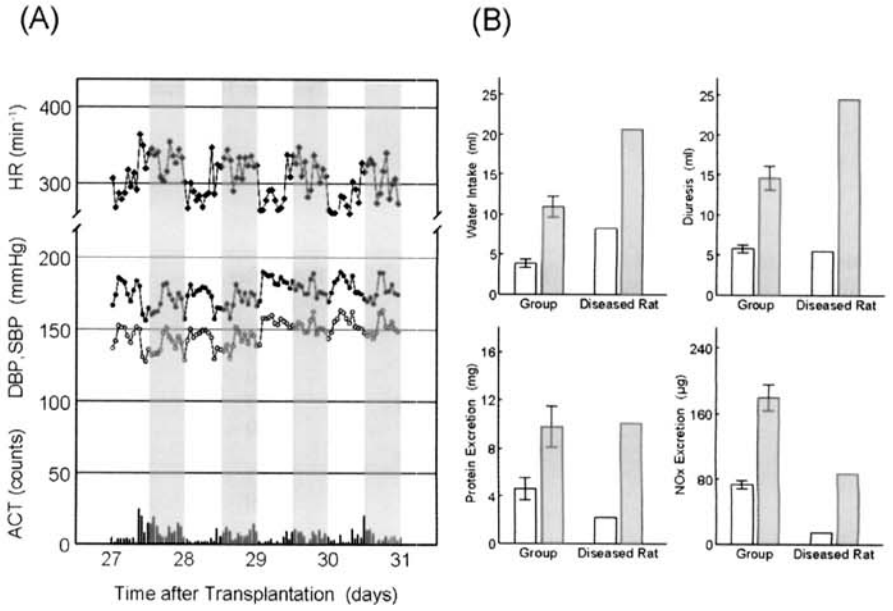

Fig. 4 Cardiovascular 24-h pattern (**A**) and day–night changes in urinary excretion (**B**) in a single rat with kidney graft dysfunction. In this animal locomotor activity was markedly depressed, blood pressure was clearly hypertensive, and the 24-h blood pressure rhythm was severely disturbed, with peak values occurring during the rest period. Water intake and diuresis were increased and urinary excretion of nitric oxide metabolites was reduced in this rat compared with the group of successfully transplanted animals ($n = 5$)

development of chronic graft rejection, and blood-pressure monitoring was carried out in the absence of cyclosporine treatment. The preserved 24-h blood-pressure pattern in the animals in the present study suggests that cyclosporine treatment may indeed represent the major factor contributing to blood-pressure changes after kidney transplantation.

While the animals that had successfully undergone transplantation remained normotensive, one rat developed severe hypertension accompanied by an inversion of the 24-h blood-pressure rhythm, and the post-mortem analysis showed hydronephrosis and signs of uremic peritonitis. The cardiovascular changes observed in this animal go well with those in human end-stage renal failure [10], and demonstrate the usefulness of telemetric blood-pressure monitoring in rat models of renal disease.

In addition to the cardiovascular variables, we also studied the circadian time-dependency of renal excretory function. Shortly after transplantation, all parameters except sodium excretion showed normal day–night patterns, with higher excretion rates in the nocturnal activity period, similar to those observed in healthy rats [7, 21, 24]. The lack of rhythmicity in sodium excretion can be attributed to the fact that rats in the present study were starved during the 24-h urine sampling, and the diurnal rhythm of sodium excretion is known to depend on the temporal pattern of food intake [7]. Consequently, our data also show that the day–night pattern in the urinary excretion of potassium, protein and nitric oxide metabolites does not simply reflect the changes in food intake, but must be due to other factors, e.g., rhythms in renal perfusion and/or glomerular filtration [9, 21]. At day 180 after transplantation, day–night variation was lost for diuresis and the excretion of creatinine, protein and nitric oxide metabolites, while potassium remained 'rhythmic'. This finding suggests that the urinary excretion of potassium may be linked most closely to the endogenous circadian clock, presumably mediated by the well-known circadian rhythms in adrenal steroid hormones.

Regarding long-term changes in renal function, it is of interest to note that proteinuria showed an increase from 24 to 180 days post-transplantation, as expected in this model of chronic graft nephropathy, while NOx excretion declined. Because creatinine excretion was preserved until the end of the observation period, the progressive reduction in NOx excretion cannot be attributed to a decrease in the glomerular filtration rate. It is tempting to speculate that the decline in NOx excretion could indicate a reduced vascular expression of endothelial NO synthase (eNOS), as observed in human renal allograft rejection [1], because a reduced availability of endothelium-derived NO may contribute to disturbed perfusion of the kidney graft and, consequently, to chronic rejection in the F344 → LEW kidney transplantation model. However, future studies will have to show whether the decrease in urinary NOx after allogeneic kidney transplantation is indeed due to downregulation of renal vascular eNOS expression.

In conclusion, the present study demonstrates that blood pressure and its 24-h pattern are preserved in a rat model of chronic kidney allograft rejection. This finding confirms clinical observations that immunosuppressive treatment with cyclosporine rather than kidney transplantation and graft nephropathy is mainly responsible for the disturbed circadian blood-pressure regulation in renal transplant recipients. Moreover, our study shows that long-term monitoring of cardiovascular parameters by telemetry is feasible in rats after experimental kidney transplantation, and can be used in order to gain more insight into the mechanisms involved in cardiovascular complications of renal graft nephropathy.

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