Kidney International, Vol. 65 (2004), pp. 1782–1789

VASCULAR BIOLOGY – HEMODYNAMICS – HYPERTENSION

Reduced endothelin-1– and nitric oxide–mediated arteriolar tone in hypertensive renal transplant recipients

JENS PASSAUER, GRIT LÄSSIG, ECKHART BÜSSEMAKER, FRANK PISTROSCH, and PETER GROSS

Division of Nephrology, Department of Medicine, University Hospital Carl Gustav Carus, Dresden, Germany

Reduced endothelin-1– and nitric oxide–mediated arteriolar tone in hypertensive renal transplant recipients.

Background. The prevalence of hypertension is high in renal transplant recipients. It has been suggested that calcineurin inhibitors (CI) contribute to the development of post-transplant hypertension by stimulating endothelin (ET-1)-mediated and/or reducing nitric oxide (NO)-mediated vascular tone.

Methods. We tested this hypothesis using 2 groups of renal transplant recipients [normotensive patients without a need for antihypertensive medication (Normo-Tx), and hypertensive patients requiring antihypertensives (Hyper-Tx)] in the presence of CI-based immunosuppression. In addition, we studied matched control subjects (C). BQ 123 (ET-A receptor antagonist), BQ123 + BQ788 (ET-A/B-receptor antagonist), ET-1, L-NMMA (NO-synthase inhibitor), acetylcholine (ACH; endothelium-dependent vasodilator), glyceroltrinitrate (GTN, NO donor), and norepinephrine (NE, endothelium-independent vasoconstrictor) were infused into the brachial artery. Forearm blood flow (FBF) was measured by venous occlusion plethysmography.

Results. Endothelium-independent vasomotion in response to GTN and NE was similar in all groups. Vascular responses to selective and combined blockade of ET receptors in both Normo-Tx and Hyper-Tx did not exceed those of C. In fact, we observed a significantly lower increase in FBF after BQ 123 ($P = 0.03$), as well as after BQ 123/788 ($P = 0.03$) in Hyper-Tx compared with Normo-Tx. This was associated with an increased vascular sensitivity to exogenous ET-1 in Hyper-Tx compared with Normo-Tx ($P = 0.04$). Vasoconstriction after L-NMMA was reduced in Hyper-Tx compared with Normo-Tx ($P = 0.015$), while the response to ACH was reduced in both groups of Tx patients to a similar degree ($P = 0.005$ vs. C).

Conclusion. Our results do not support a major role for the vascular endothelin system in the hypertension of renal transplant recipients, whereas deficient baseline NO production may be a contributing factor.

Hypertension is a frequent abnormality in kidney transplant recipients. Hypertension is also a known risk factor for cardiovascular mortality and for renal transplant survival [1]. The causes of this hypertension are not fully understood. It has been proposed that the recipient's own kidneys, the blood pressure of the donor, glucocorticoid medication, calcineurin inhibitors (CI), and other factors are involved. Calcineurin inhibitors are known to stimulate the generation of endothelin-1 (ET-1) in vitro and in vivo [2, 3]. Several lines of evidence suggest that ET-1 plays a pivotal role in post-transplant hypertension [4–6]. In addition, it was reported that calcineurin inhibitors may interfere with nitric oxide (NO)-mediated vascular relaxation [7–9].

We were therefore interested in the question if increased vascular ET-1 and/or reduced nitric oxide could be shown to participate in post-transplant hypertension–associated vascular dysfunction in patients. We tested the functional state of the vascular endothelin and NO system in vivo in renal allograft recipients with hypertension and matched healthy control subjects. We studied arterial resistance vessels of the forearm circulation, a representative vascular bed for testing blood pressure regulation. We asked the following specific questions: (*1*) is there evidence for a general increase in ET-1 mediated arteriolar tone in Tx patients with calcineurin inhibitor-based immunosuppression? and (*2*) is there an imbalance between ET-1– and NO-mediated vascular tone specifically in those Tx patients who require antihypertensive medication?

We used intrabrachial infusions of selective (ET-A receptor), as well as of unselective (ET-A/B) endothelin receptor antagonism. We also tested the vascular effects of ET-1 itself. Furthermore, we measured the arteriolar responses to blockade of NO synthases (NOS), as well as to stimulation of endothelial NOS (eNOS). Finally, we determined endothelium-independent vasoconstriction and vasodilation.

METHODS

Subjects

The study was approved by the institutional review board of our hospital. All participants gave written informed consent.

Received for publication February 28, 2003
and in revised form July 7, 2003, and October 12, 2003
Accepted for publication November 20, 2003

Renal transplant recipients who received calcineurin inhibitors and had excellent graft function were included. Only patients whose transplantation had been performed more than 12 months ago were included in the studies. Patients with diseases potentially interfering with endothelial function (diabetes mellitus, congestive heart failure, liver cirrhosis, manifested vascular diseases) were excluded. All patients were nonsmokers. Tx patients were divided into two groups: (*1*) patients with normal blood pressure (Normo-Tx) not requiring antihypertensive medication. The normotensive state was verified by 24-hour blood pressure recording; (*2*) patients with hypertension. These patients were taking at least two different antihypertensive agents for adequate blood pressure control (Hyper-Tx). Patients of both groups (Normo-Tx, Hyper-Tx) were selected in such a way that they were matched for age, gender, height, body weight, and serum cholesterol, as well as for the intake of statins and the type of calcineurin inhibitor used [cyclosporine A (CSA) or tacrolimus (FK)].

Control subjects were asked for their past medical history and received a physical examination.

In Tx patients studies were performed during the first four hours after the intake of their morning dose of calcineurin inhibitor. In the Hyper-Tx group antihypertensive medication was withdrawn before the studies. Patients were instructed to take a final dose of antihypertensive drugs 36 hours before each experimental session. This relatively short washout period was chosen because the different protocols of infusion were separated by 7 days only. A longer period of discontinuation of antihypertensives appeared to be inappropriate for ethical reasons. All participants were asked to refrain from large meals and from beverages containing alcohol or caffeine during 6 hours before the start of each protocol.

Technique

Studies were performed in a quiet, temperature-controlled room (23 to 25°C) with the subjects resting supine. In control subjects the brachial artery of the nondominant arm (in Tx patients the arm without the arteriovenous fistula) was cannulated by a 27G steel needle (Cooper Needleworks, Birmingham, UK) for drug infusion. Forearm blood flow (FBF) was measured by a calibrated venous occlusion plethysmograph (Gutmann Medizinelektronik, Eurasburg, Germany), as described previously [10]. The pressure of the upper arm-congesting cuff was set to 40 mm Hg. During the measurements the circulation of the hand was occluded by a wrist cuff inflated to 220 mm Hg. FBF was measured for 10 seconds in each 15-second cycle.

Infusion protocols

The complete study consisted of four infusion protocols that were conducted on separate days at least 7 days apart. After cannulation of the brachial artery a resting period of 20 minutes was allowed before at least two baseline FBF recordings were obtained. All infusions were given at a constant rate of 1 mL/min.

Protocol 1. BQ 123 (ET-A receptor antagonist) was infused at 40 nmol/min for 60 minutes. FBF readings were obtained every 10 minutes.

Protocol 2. BQ 123 at 40 nmol/min was infused together with BQ 788 (ET-B receptor antagonist) at 50 nmol/min for 60 minutes. FBF was recorded every 10 minutes.

Protocol 3. Norepinephrine (NE) was infused in 3 increasing doses (60, 120, and 240 nmol/min, each dose for 5 min). FBF was recorded during the last 2.5 minutes of each infusion period. After 30 minutes of saline infusion ET-1 at 5 pmol/min was given for 60 minutes. FBF readings were performed every 10 minutes.

Protocol 4. Venous blood was drawn for determination of plasma levels of ET-1. Acetylcholine (ACH) was infused in 3 increasing doses (55, 110, and 220 nmol/min; each dose over 5 min). After a resting period of 30 minutes N-monomethyl-L-arginine (L-NMMA) was given at 1, 2, and 4 μmol/min. Another resting period of 30 minutes was followed by infusion of GTN at 2.2, 4.4, and 8.8 nmol/min. FBF was recorded during the last 2.5 minutes of each period of drug infusion.

Drugs and agents

All solutions of drugs and agents were prepared fresh before each protocol with isotonic saline to obtain the required concentrations. Drugs and agents were obtained as follows: BQ 123, BQ 788, ET-1, and L-NMMA from Clinalfa (Läufelfingen, Switzerland); ACH as Miochol-E® from Ciba Vision (Germering, Germany); GTN as Perlinganit® (Schwarz Pharma, Monheim, Germany); and NE as Arterenol® (Aventis, Frankfurt, Germany).

Calculations and statistics

A determination of FBF consisted of 10 repetitive individual measurements of FBF, and was calculated as the mean of the last 5 measurements. Data are expressed as the percent change from baseline FBF (with baseline set to 0%) ± standard error of mean (SEM) as calculated by the formula:

FBF (% change) = [(FBF observed/baseline FBF) × 100] − 100.

Comparisons between groups were performed by two-way analysis of variance (ANOVA) for repeated measurements with the computer software Statistical Package for the Social Sciences (SPSS) version 10.0.1 (Chicago, IL, USA). A $P < 0.05$ was considered significant.

RESULTS

The studies were tolerated well by all participants. There were no side effects from arterial puncture or drug infusion. We enrolled a total of 20 renal transplant recipients (9 Normo-Tx and 11 Hyper-Tx) and 30 healthy

Table 1. Group characteristics

	Characteristic	Control	Normo-Tx	Hyper-Tx	*P* value (Normo-Tx vs. Hyper-Tx)
Protocol 1	*N*	18	8	10	
	Male/female	15/3	6/2	7/3	
	Age *years*	33 ± 2	36 ± 4	42 ± 3	0.22
	Height *cm*	175 ± 2	171 ± 4	174 ± 3	0.58
	Weight *kg*	71 ± 3	71 ± 4	76 ± 3	0.27
	Blood pressure *mm Hg*				
	Systolic	120 ± 1	126 ± 2	148 ± 3	<0.0001
	Diastolic	75 ± 2	78 ± 2	90 ± 3	<0.0001
	Cholesterol *mmol/L*	4.8 ± 0.1	5.0 ± 0.2	5.1 ± 0.2	0.75
	Creatinine µ*mol/L*	76 ± 6	133 ± 10	130 ± 8	0.52
	CSA/FK		4/4	5/5	
	Statins		*N* = 2	*N* = 4	
Protocol 2	*N*	15	8	9	
	Male/female	13/2	6/2	6/3	
	Age *years*	33 ± 2	36 ± 4	43 ± 3	0.11
	Height *cm*	176 ± 3	171 ± 4	174 ± 3	0.54
	Weight *kg*	72 ± 3	71 ± 4	78 ± 3	0.21
	Blood pressure *mm Hg*				
	Systolic	121 ± 2	125 ± 3	151 ± 4	<0.0001
	Diastolic	75 ± 2	79 ± 1	89 ± 2	<0.0001
	Cholesterol *mmol/L*	4.8 ± 0.5	5.0 ± 0.7	5.2 ± 0.7	0.73
	Creatinine µ*mol/L*	78 ± 7	136 ± 11	131 ± 8	0.65
	CSA/FK		4/4	4/4	
	Statins		*N* = 2	*N* = 4	
Protocol 3	*N*	11	8	8	
	Male/female	8/3	6/2	5/3	
	Age *years*	40 ± 4	36 ± 4	44 ± 3	0.09
	Height *cm*	172 ± 2	171 ± 4	173 ± 3	0.71
	Weight *kg*	70 ± 4	71 ± 4	79 ± 3	0.11
	Blood pressure *mm Hg*				
	Systolic	120 ± 3	126 ± 2	153 ± 4	<0.0001
	Diastolic	75 ± 2	77 ± 2	87 ± 2	<0.0001
	Cholesterol *mmol/L*	4.9 ± 0.2	5.0 ± 0.2	5.3 ± 0.2	0.52
	Creatinine µ*mol/L*	73 ± 5	130 ± 11	128 ± 9	0.61
	CSA/FK		4/4	5/3	
	Statins		*N* = 2	*N* = 4	
Protocol 4	*N*	10	9	7	
	Male/female	5/3	5/4	5/2	
	Age *years*	39 ± 4	40 ± 5	41 ± 5	0.87
	Height *cm*	169 ± 3	167 ± 2	172 ± 3	0.18
	Weight *kg*	66 ± 4	68 ± 4	76 ± 4	0.18
	Blood pressure *mm Hg*				
	Systolic	123 ± 3	124 ± 2	149 ± 4	<0.0001
	Diastolic	77 ± 2	73 ± 2	87 ± 2	<0.0001
	Cholesterol *mmol/L*	5.0 ± 0.1	5.0 ± 0.3	5.1 ± 0.2	0.68
	Creatinine µ*mol/L*	78 ± 4	136 ± 12	129 ± 7	0.72
	CSA/FK		5/4	5/2	
	Statins		*N* = 3	*N* = 4	

CSA, cyclosporin A; FK, tacrolimus.

control subjects. Not all participants completed all four protocols. Therefore, the baseline characteristics of study patients and control subjects are given separately for each protocol in Table 1. Patients of the Hyper-Tx group had the following antihypertensive medication: β-blockers ($N = 11$), diuretics ($N = 5$), calcium antagonists ($N = 8$), sympatholytic agents ($N = 5$), angiotensin-converting enzyme inhibitors ($N = 2$), and angiotensin II receptor antagonists ($N = 1$).

Plasma levels of ET-1 were measured once in all participants in protocol 4. They were significantly elevated in Tx patients (6.5 ± 0.4 pmol/L in Normo-Tx and 7.9 ± 1.4 in Hyper-Tx) compared with control subjects (4.0 ± 0.3; $P = 0.005$ C vs. Normo-Tx, $P = 0.63$ Normo-Tx vs. Hyper-Tx).

Protocol 1 (C: $N = 18$, Normo-Tx: $N = 8$, Hyper-Tx: $N = 10$). Infusions of BQ 123 resulted in a time-dependent increase in FBF in all groups. Vascular responses of Hyper-Tx were significantly reduced compared with Normo-Tx ($P = 0.03$) (Table 2, Fig. 1A), while those in C and Normo-Tx were comparable.

Protocol 2 (C: $N = 15$, Normo-Tx: $N = 8$, Hyper-Tx: $N = 9$). Combined blockade of both ET-A and ET-B receptors significantly increased FBF in all groups. Within all groups the magnitude of this change in FBF tended to be more pronounced than with ET-A receptor blockade

Table 2. FBF data in absolute values (mL/dL × min) and in percent change from baseline (baseline = 0%) of all experimental protocols

	FBF	Control	%	Normo-Tx	%	Hyper-Tx	%	*P* value Normo-Tx vs. Hyper-Tx
Protocol 1	Baseline	2.5 ± 0.3	0	2.7 ± 0.3	0	3.0 ± 0.6	0	0.67
	BQ123 10`	2.8 ± 0.3	16 ± 5	3.3 ± 0.4	19 ± 6	3.3 ± 0.9	3 ± 6	
	20`	3.0 ± 0.4	23 ± 11	3.9 ± 0.5	48 ± 12	3.4 ± 0.9	3 ± 7	0.03
	30`	3.4 ± 0.5	41 ± 13	4.3 ± 0.5	60 ± 15	3.6 ± 1.0	9 ± 9	ANOVA
	40`	3.8 ± 0.5	52 ± 14	4.4 ± 0.7	57 ± 19	3.9 ± 1.0	18 ± 11	
	50`	3.9 ± 0.5	60 ± 17	4.5 ± 0.7	65 ± 20	3.8 ± 1.0	15 ± 10	
	60`	4.2 ± 0.6	74 ± 23	4.3 ± 0.8	56 ± 22	3.8 ± 1.0	12 ± 12	
Protocol 2	Baseline	2.4 ± 0.3	0	2.7 ± 0.5	0	2.7 ± 0.5	0	0.96
	BQ123/788 10`	2.8 ± 0.4	18 ± 11	3.6 ± 0.6	32 ± 7	3.2 ± 0.6	17 ± 9	
	20`	3.3 ± 0.5	45 ± 14	3.9 ± 0.7	45 ± 10	3.4 ± 0.7	22 ± 9	0.03
	30`	3.8 ± 0.5	63 ± 21	4.6 ± 0.8	73 ± 9	3.8 ± 1.0	31 ± 17	ANOVA
	40`	4.2 ± 0.6	89 ± 24	5.0 ± 0.9	85 ± 13	3.9 ± 1.0	36 ± 16	
	50`	4.7 ± 0.6	114 ± 32	5.0 ± 0.9	91 ± 15	3.8 ± 1.0	35 ± 15	
	60`	4.8 ± 0.6	115 ± 26	4.8 ± 0.9	81 ± 21	3.9 ± 1.1	35 ± 19	
Protocol 3	Baseline	2.4 ± 0.2	0	3.2 ± 0.3	0	3.3 ± 0.6	0	0.92
	NE 60 *pmol/min*	1.9 ± 0.2	−20 ± 3	2.5 ± 0.3	−24 ± 3	2.7 ± 0.5	−19 ± 5	
	120 *pmol/min*	1.8 ± 0.3	−28 ± 5	2.3 ± 0.4	−32 ± 7	2.2 ± 0.4	−35 ± 5	0.68
	240 *pmol/min*	1.5 ± 0.2	−39 ± 6	1.8 ± 0.3	−43 ± 5	1.9 ± 0.4	−43 ± 7	ANOVA
	Baseline	2.8 ± 0.4	0	3.3 ± 0.4	0	3.0 ± 0.6	0	0.743
	ET-1 10`	2.6 ± 0.3	−6 ± 6	3.0 ± 0.4	−6 ± 6	2.3 ± 0.5	−27 ± 7	
	20`	2.3 ± 0.2	−16 ± 6	2.8 ± 0.4	−11 ± 8	2.2 ± 0.5	−32 ± 8	0.04
	30`	2.2 ± 0.2	−18 ± 7	2.9 ± 0.4	−8 ± 9	2.1 ± 0.4	−32 ± 8	ANOVA
	40`	2.0 ± 0.2	−28 ± 6	2.5 ± 0.4	−23 ± 6	2.0 ± 0.4	−34 ± 6	
	50`	1.8 ± 0.2	−31 ± 6	2.7 ± 0.5	−16 ± 10	1.9 ± 0.4	−40 ± 8	
	60`	1.6 ± 0.2	−41 ± 4	2.6 ± 0.5	−24 ± 10	1.8 ± 0.4	−43 ± 9	
Protocol 4	Baseline	2.4 ± 0.2	0	1.8 ± 0.3	0	3.1 ± 0.8	0	0.18
	ACH 55 nmol/min	10.9 ± 1.3	344 ± 60	6.3 ± 1.0	302 ± 73	8.6 ± 1.6	258 ± 71	0.005[a]
	110 nmol/min	15.0 ± 2.2	506 ± 83	7.2 ± 0.9	368 ± 78	11.4 ± 2.0	413 ± 132	ANOVA
	220 nmol/min	21.4 ± 3.2	784 ± 108	8.4 ± 1.1	459 ± 107	11.8 ± 2.1	388 ± 118	
	Baseline	3.5 ± 0.4	0	2.4 ± 0.4	0	3.0 ± 0.6	0	0.47
	L-NMMA 1 μmol/min	2.8 ± 0.4	−21 ± 5	1.8 ± 0.3	−26 ± 3	2.5 ± 0.8	−16 ± 6	0.015
	2 μmol/min	2.3 ± 0.4	−37 ± 5	1.5 ± 0.2	−39 ± 4	2.2 ± 0.7	−28 ± 7	ANOVA
	4 μmol/min	1.7 ± 0.2	−52 ± 4	1.0 ± 0.2	−55 ± 4	2.0 ± 0.6	−34 ± 3	
	Baseline	2.3 ± 0.3	0	1.7 ± 0.2	0	2.5 ± 0.8	0	0.39
	GTN 2.2 nmol/min	4.1 ± 0.6	92 ± 21	3.1 ± 0.3	86 ± 11	4.8 ± 1.2	130 ± 28	0.23
	4.4 nmol/min	5.6 ± 0.7	156 ± 26	3.8 ± 0.5	130 ± 18	5.8 ± 1.5	181 ± 38	ANOVA
	8.8 nmol/min	7.5 ± 0.8	245 ± 30	4.9 ± 0.8	186 ± 24	7.0 ± 1.7	253 ± 58	

Statistical comparisons are focused on differences between Normo-Tx and Tx HBP, and include those of baseline FBF values before each protocol (in mL/dL × min by *t* test), as well as comparisons of experimental data within each protocol (in % change from baseline by ANOVA for repeated measurements).
[a] Control vs. Normo-Tx.

alone, although the differences did not reach significance. The increase in FBF was significantly less in Hyper-Tx compared with Normo-Tx ($P = 0.03$) (Table 2, Fig. 1B).

Protocol 3 (C: N = 11, Normo-Tx: N = 8, Hyper-Tx: N = 8). Infusion of NE reduced FBF in all groups in a comparable manner (Table 2, Fig. 3B).

Infusion of ET-1 was followed by a time-dependent decrease of FBF in all participants. The reduction in FBF was significantly less in Normo-Tx compared with Hyper-Tx ($P = 0.04$) (Table 2, Fig. 1C).

Protocol 4 (C: N = 10, Normo-Tx: N = 9, Hyper-Tx: N = 7). ACH increased FBF in a dose-dependent manner. The increase in FBF was comparable in Normo-Tx and Hyper-Tx, but significantly reduced compared with C ($P = 0.005$ C vs. Normo-Tx) (Table 2, Fig. 2B).

L-NMMA dose-dependently reduced FBF in all groups. The decrease in FBF was significantly less in Hyper-Tx compared with Normo-Tx ($P = 0.015$) (Table 2, Fig. 2A).

GTN increased FBF in all groups comparably (Table 2, Fig. 3A).

In all participants who completed both protocol 2 and protocol 3 we calculated the ratio of ET-1–mediated vascular tone (individual maximum effect of unselective ET-receptor blockade) to ET receptor sensitivity (individual maximum effect of ET-1 itself) as an estimate of effective endogenous ET-1 production (Fig. 4). In Hyper-Tx effective ET-1 production was significantly reduced compared with Normo-Tx ($P = 0.015$).

To examine the individual balance between ET-1– and NO-mediated vascular tone in both groups of renal transplant recipients we created a scatter plot of the maximum effects of combined ET receptor blockade versus the maximum effects of NO synthase inhibition in all subjects having completed protocols 2 and 4 (Fig. 5). Data of the control group are not shown because only four subjects completed both protocols 2 and 4.

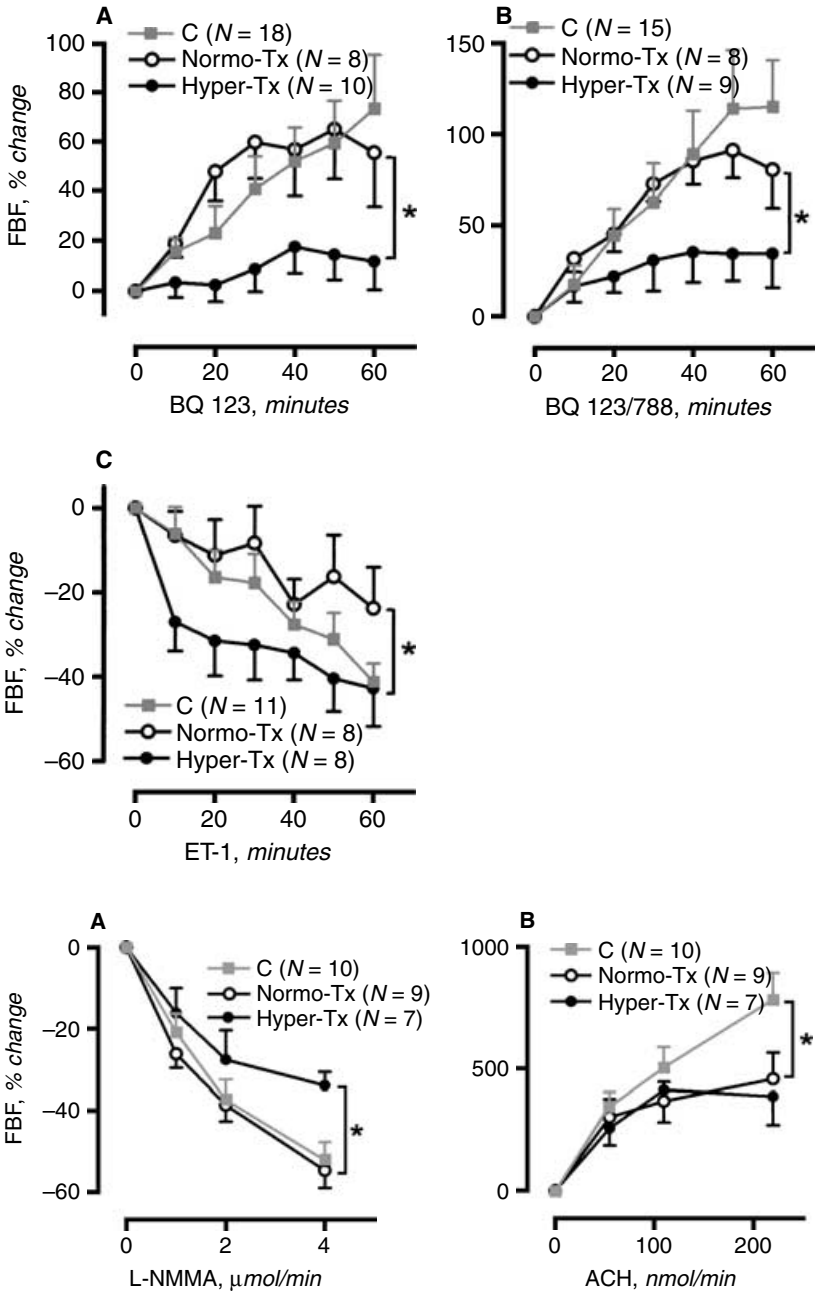

Fig. 1. Forearm blood flow (FBF) (% change from baseline, baseline = 0%) in response to intrabrachial infusions of (*A*) BQ 123 (40 nmol/min), (*B*) BQ 123/788 (40/50 nmol/min), and (*C*) endothelin-1 (ET-1 5 pmol/min) in control subjects (C), as well as normotensive (Normo-Tx) and hypertensive (Hyper-Tx) renal transplant recipients. Statistical differences between Normo-Tx and Hyper-Tx are shown. $^*P < 0.05$.

Fig. 2. (*A*) Baseline endothelial nitric oxide generation was assessed by graded infusions of L-NMMA (1, 2, and 4 μmol/min). (*B*) Endothelium-dependent vasodilation in response to acetylcholine (ACh, 55, 110, and 220 nmol/min). $^*P < 0.05$.

DISCUSSION

This study was designed to describe the functional state of the vascular ET—and NO—systems in renal transplant recipients maintained on a calcineurin inhibitor–based immunosuppressive regimen. Before discussing our main results, comments on the role of ET in the regulation of vascular tone in healthy subjects, as well as on methodologic aspects of our study may be appropriate.

In the literature there is prevailing consensus that endogenous ET-1 mediates a basal vasoconstrictor tone in healthy humans [11, 12]. ET-A receptors located on vascular smooth muscle cells mediate a major part of the constrictor properties of ET-1. Endothelial ET-B receptors mediate vasodilation via NO, whereas ET-B receptors located on smooth muscle cells cause vasoconstriction. In addition, the ET-B receptor acts as a clearance receptor for ET-1. Selective blockade of ET-B may thus result in an increased binding of ET-1 to ET-A receptors. We therefore combined ET-A and ET-B receptor blockade and did not perform experiments with selective blockade of ET-B receptors.

Currently conflicting results exist about the hemodynamic net effect of ET-B receptor blockade in healthy subjects. This may be related to different doses of BQ 788

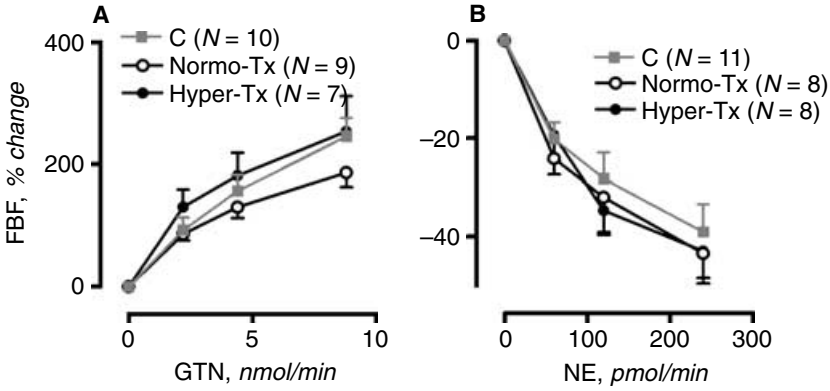

Fig. 3. (*A*) Endothelium-independent vasodilation in response to glyceroltrinitrate (GTN, 2.2; 4.4, and 8.8 nmol/min), and (*B*) endothelium-independent vasoconstriction in response to norepinephrine (NE; 60, 120, and 240 pmol/min).

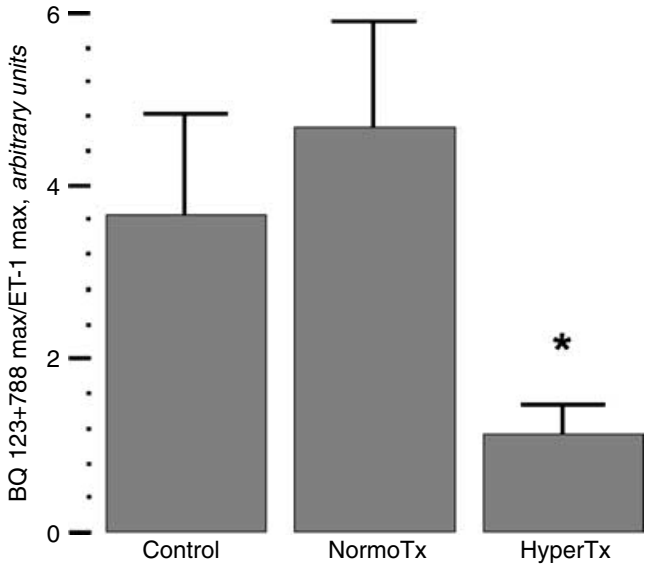

Fig. 4. Estimation of effective vascular endothelin-1 (ET-1) production in the forearm based on functional data. The ratio between the individual maxima of ET-1 mediated vascular tone (response to BQ 123 + 788) and ET-1 sensitivity (response to ET-1 itself, absolute values) was calculated for control ($N = 6$), Normo-Tx($N = 8$), and Hyper-Tx($N = 8$). $^*P = 0.015$ Normo-Tx vs. Hyper-Tx.

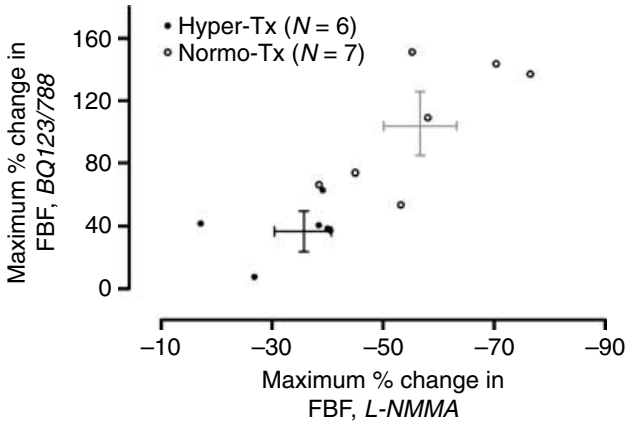

Fig. 5. Individual balance of nitric oxide (NO)- and endothelin (ET)-1–mediated vascular tone in renal transplant recipients. In Hyper-Tx, contribution of both NO and ET-1 to baseline vascular tone is reduced compared with Normo-Tx. Error bars represent mean ± SD.

used. In the study of Verhaar et al [11] the net effect of ET-B blockade by BQ 788 at 1 nmol/min was vasoconstriction. This dose had been adopted from hand vein experiments. Based on pharmacologic consideration Cardillo et al [13] proposed a 50-fold higher dose of BQ 788 for forearm blood flow studies. We have recently shown that this high dose of BQ 788 further enhances vasodilation elicited by complete inhibition of ET-A receptors, indicating a role for ET-B receptors located on myocytes, which are possibly not inhibited by low-dose BQ 788 (discussed in detail in [14]). Because up-regulation of ET-B on myocytes may play a role in Tx patients we kept the dose of BQ 788 at 50 nmol/min for the present study.

Undoubtedly, bilateral plethysmography is the preferable method for testing the influence of drugs and mediators on forearm blood flow [15–17]. However, all Tx patients enrolled in our studies had patent arteriovenous fistulas on one arm, which makes bilateral plethysmography impossible. In the control group we used the two-arm approach. Comparison between bilateral and unilateral plethysmography within this group revealed no significant differences between both methods (data not shown). In view of the constancy of blood pressure and heart rate throughout the experiments it is unlikely that systemic effects of the drugs infused biased our results.

Several studies demonstrated in vitro that calcineurin inhibitors are able to increase endothelial ET-1 production [3]. In our study plasma levels of ET-1 were significantly elevated in Tx patients compared with control subjects, which is in line with previous reports [2]. However, one major result is that in Tx patients the vascular ET system is not overactive compared with matched control subjects. This is primarily shown by the fact that vascular responses to both selective and unselective ET receptor blockade in either group of Tx patients exceeded that of control subjects (Fig. 1A and B). In this way our results support the broadly accepted view that ET-1

plasma concentrations do not reflect ET-1–mediated vascular tone [14, 18–20]. Published data suggest that the elevated ET-1 plasma concentrations of Tx patients are unable to cause significant vasoconstriction, per se. Intrabrachial infusion of ET-1 at 0.5 ng/min had no effect on forearm blood flow in healthy volunteers [21]. This dose can be expected to increase the ET-1 plasma level in the forearm by 2 to 4 pmol/L, which approximates the differences in ET-1 plasma concentration between control and Tx patients in our study.

We observed differences in the vascular responsiveness to ET-1 and ET receptor antagonists between hypertensive and normotensive renal transplant recipients. Hypertensive patients showed a higher vascular sensitivity to exogenously administered ET-1 than normotensive patients. Similar observations have been reported in patients with essential hypertension [13, 22]. Surprisingly, the increased vascular sensitivity to ET-1 in hypertensive patients was not associated with an increase in ET-1–mediated arteriolar tone in this group. In fact, the vascular responses to ET receptor blockade were significantly reduced in Hyper-Tx (whereas responses of Normo-Tx were mostly at control level). These differences cannot be explained by ET receptor down-regulation because the sensitivity to exogenous ET-1 in Hyper-Tx was even increased. Potential confounding factors like kidney function [14, 19], intake of statins [23], as well as kind and plasma level of calcineurin inhibitors were excluded by our matching of patients. To estimate the vascular ET-1 production in the forearm we have calculated the ratio of ET-1–mediated vascular tone (maximum effect of unselective ET receptor blockade) to ET- receptor sensitivity (maximum effect of ET-1 itself) for each group. As this ratio was significantly reduced in Hyper-Tx compared with Normo-Tx we hypothesize a reduced vascular production of ET-1 in Hyper-Tx. However, further experiments are needed to verify this hypothesis. Furthermore, we cannot fully rule out that antihypertensive medication may have influenced the pattern of vascular responses in Hyper-Tx. In this respect, the time between withdrawal of antihypertensive medication and forearm blood flow studies may have been too short for complete washout of drugs with long half-lives. However, all Hyper-Tx patients experienced an increase in blood pressure after withdrawal of antihypertensives, and had moderate hypertension at the time of FBF measurements. We therefore conclude that endogenous ET-1 does not substantially contribute to the constrictor tone of forearm resistance vessels in Hyper-Tx.

Hypertensive patients had a lower baseline NO-mediated vascular tone than normotensive patients. This reduced baseline NO may be an important factor contributing to hypertension in Hyper-Tx. Possible explanations for the coincidence of low baseline NO and increased sensitivity to ET-1 in Hyper-Tx include modulating effects of NO on ET receptors. Based on their in vitro studies Wiley et al [24] suggested that NO was able to reverse ET-1–induced vasoconstriction by direct interaction with the ET receptor.

By plotting the individual maxima of vascular response to L-NMMA (NO-mediated vascular tone) against the maxima of vascular response to BQ123/788 (ET-1–mediated vascular tone) we can demonstrate that both NO- and ET-1–mediated vascular tone are reduced in Hyper-Tx compared with Normo-Tx (Fig. 5). This finding argues against the assumption that in hypertensive patients the vascular balance between NO and ET-1 might be shifted toward the latter.

Both groups of renal transplant recipients showed an equal impairment of vascular response to ACH compared with control, indicating endothelial dysfunction. Similar results have been reported in the literature [7, 25, 26].

CONCLUSION

Kidney transplantation with concomitant calcineurin inhibitor–based immunosuppression is not associated with a general increase in ET-1–mediated vascular tone. Moreover, in the forearm circulation of hypertensive renal transplant recipients the vascular endothelin system is not overactive, but suppressed. In these patients reduced baseline NO may contribute to hypertension. Our results, however, do not fully exclude a role for ET-1 in post-transplant hypertension because our findings were obtained in the forearm circulation and did not consider renal effects of ET-1. Moreover, endothelin antagonists may influence vascular and cardiac remodeling, as well as chronic allograft rejection, which we have not tested. Long-term studies using systemic doses of endothelin antagonists are required to clarify these issues.

Reprint requests to Dr. Jens Passauer, Universitätsklinikum Carl Gustav Carus, Technische Universität Dresden, Medizinische Klinik III/Nephrologie, Fetscherstr. 74, 01307 Dresden, Germany.
E-mail: passauer@rcs.urz.tu-dresden.de

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