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Endothelin as a causative factor of blunted volume reflex in diabetic rats

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1 The study investigated whether endothelin (ET) contributed to the diabetes-associated alterations in volume reflex and characterised the receptor subtype that might be involved. The influence of renal sympathetic nerves on these aspects of ET was also examined.

2 Groups of nondiabetic and streptozotocin-induced diabetic rats were subjected to an acute isotonic saline volume expansion (VE), 10% body wt in the presence and absence of ET antagonists.

3 Cumulative urine sodium excretion ($CuU_{Na}V$) after VE in diabetic rats reached values of 116 ± 10 in the denervated and 74 ± 6 μmol min^{-1} g kidney wt^{-1} in the innervated kidneys, which were both less (both $P<0.001$) than those achieved in the nondiabetic rats, at 267 ± 9 in the denervated and 183 ± 10 μmol min^{-1} g kidney wt^{-1} in the innervated kidney, respectively.

4 Diabetic rats pretreated with a nonselective ET_A/ET_B antagonist had an enhanced $CuU_{Na}V$ in the denervated kidneys by 37% ($P<0.01$) compared to that of untreated diabetic rats. At both doses of SB209670 these increments were less than the values obtained previously in nondiabetic rats (both $P<0.01$). The ET_A/ET_B antagonist had no meaningful effect on $CuU_{Na}V$ in the innervated kidneys of the diabetic rats, whereas previous studies in nondiabetic rats showed the response to be depressed. The $CuU_{Na}V$ responses to VE in diabetic rats given the selective ET_A antagonist were not different from those observed in untreated diabetic rats, irrespective of whether or not the renal nerves were present. In nondiabetic rats, the ET_A antagonist had an action similar to the mixed antagonist.

5 These findings demonstrate that activation of ET_B receptors contributes to the depressed ability to excrete a saline load in diabetes mellitus, but its impact is obscured by the influence of the renal nerves. *British Journal of Pharmacology* (2003) **138,** 1403–1410. doi:10.1038/sj.bjp.0705133

Keywords: Endothelin; endothelin antagonists; volume expansion; renal nerves; diabetes mellitus

Abbreviations: CuUV, cumulative urine flow; $CuU_{Na}V$, cumulative urine sodium excretion; ET, endothelin; FENa, fractional sodium excretion; STZ, streptozotocin; UV, urine flow; $U_{Na}V$, absolute urine sodium excretion; VE, volume expansion

Introduction

Altered fluid balance and increased exchangeable sodium are important causal factors in diabetic hypertension (Sower & Epstein, 1995; Patel, 1997). There may be a number of pathways that participate in this deranged volume and sodium homeostasis, but a disturbance in the volume reflex, a reflex that defends the body against changes in extracellular fluid volume, has been proposed. This possibility is based on a substantial body of evidence, which demonstrated that both diabetic animals and humans have an impaired ability to excrete an acute saline load (Patel & Zhang, 1989; Zhang *et al*., 1991; Beretta-Piccoli *et al*., 1994; Patel *et al*., 1997). The deficits in the reflex fall into three main categories; a failure to suppress renal sympathetic nerve activity, tubular deficiencies, and an inappropriate release or response to natriuretic factors (Zhang *et al*., 1991; Patel & Zhang, 1994; Patel *et al*., 1997; Wongmekiat & Johns, 2001a). Nevertheless, the mechanisms involved in the altered volume reflex seen in diabetes remain uncertain and need to be examined further.

The endothelins (ET) occur as three isopeptides; endothelin-1 (ET-1), a 21-amino-acid peptide, is one of the autocrine/paracrine factors that has been highlighted as playing a significant role in various aspects of kidney functions with the other endothelins, ET-2 and ET-3 providing a smaller contribution. An increased renal production of ET has been reported in both experimental animal models and diabetic patients as demonstrated by the elevation of urinary ET-1 excretion (Morabito *et al*., 1994; Hocher *et al*., 1998). The plasma levels of ET have also been shown to be significantly elevated in diabetes, which might reflect a decreased clearance by the liver and kidneys (Hocher *et al*., 1998; Makino & Kamata, 1998). Studies using Northern blot analysis demonstrated that the mRNA for ET-1 in glomeruli of diabetic rats was increased with the progression of diabetic nephropathy (Koide *et al*., 1995). In addition, several growth factors that are increased in the diabetic state have been shown to enhance ET-1 mRNA levels in cultured mesangial cells and glomerular capillary endothelial cells (Koide *et al*., 1995). From these observations, it is conceivable that an activated ET system might contribute to the blunted volume reflex observed in the diabetic condition, but no information in this aspect is available at present.

This study set out to investigate whether endogenous ET could possibly modulate the renal haemodynamic and excretory responses to volume expansion in diabetic rats and

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to identify the ET receptor subtype that might be involved. Since diabetes mellitus has been reported to be associated with increased activity in the renal sympathetic nerves (Patel, 1997), how this tonic influence of renal nerves might modulate the renal responses to VE following infusion of ET antagonists was also examined.

Methods

All experimentation was carried out in compliance with permissions granted by Her Majesty's Government Home Office licences PPL 40/1367, PIL 40/371, and PIL 40/5301.

Diabetic induction

Diabetes mellitus was induced in male Wistar rats using a single injection of streptozotocin (STZ, Sigma Chemical, St Louis, MO, U.S.A.) 60 mg kg^{-1} into the tail vein. Two weeks after STZ injection, the development of diabetes was verified by measurement of blood glucose levels. Rats with blood glucose levels less than 10 mmol l^{-1} were excluded from the study.

Surgical procedures

All animals were anaesthetized with a mixture of fluothane and nitrous oxide in oxygen followed by an intravenous alpha-chloralose/urethane (Sigma Chemical, St Louis, MO, U.S.A.) mixture (12 and 180 mg ml^{-1}, respectively), as necessary. A tracheostomy was performed and the animals breathed spontaneously. The right femoral vein was cannulated for saline infusion and the right femoral artery for measurement of arterial blood pressure (MAP) and blood sampling. Both kidneys were approached retroperitoneally and their ureters were catheterized. A left renal denervation was performed and considered complete when the renal vasoconstrictor response to direct electrical stimulation (15 V, 10 Hz, 0.2 ms for 10 s) of the proximal renal nerve bundle was abolished. An electromagnetic flow probe (Carolina EP100 series; internal circumference 2.5 mM) was fitted around the left renal artery and connected to a square-wave flowmeter (FM 501, Carolina Medical Electronics, Inc., NC, U.S.A.) for continuous recording of renal blood flow (RBF). On completion of surgery, a 2-ml bolus of inulin (Sigma Chemical, St Louis, MO, U.S.A.) in saline (1.5 g 100 ml^{-1}) was given intravenously and then as a continuous infusion at 3 ml h^{-1} for the remainder of the experiment. The animal was allowed 2 h to recover from the experimental preparation.

Experimental series

Series 1: Renal responses to acute volume expansion The renal excretory responses to acute volume expansion were evaluated in groups of nondiabetic ($n=8$) and untreated diabetic ($n=7$) rats. Two 15-min baseline urine collections were obtained after the animal was stabilised. VE was then started by intravenous infusion of isotonic saline at 0.25% body wt min^{-1} for 40 min (10% body wt VE) with 5-min clearances being taken. Arterial blood samples were collected at the beginning, at the end of the baseline period, and at the end of VE for the determination of blood glucose, plasma inulin, and sodium. At the end of the experiment, the animals were killed with an intravenous bolus of 60 mg pentobarbital sodium (Rhone Merieux, U.K.). Both kidneys were removed, decapsulated, blotted dry, and weighed in order to standardise renal function measurements on a kidney weight basis.

Series 2: Effect of endothelin antagonist on renal responses to volume expansion This series of experiments explored the role of ET on systemic and renal haemodynamics and renal excretory function either at the basal state or in response to VE by blocking ET actions with a nonselective ET_A/ET_B receptor antagonist, SB209670 (SmithKline Beecham Pharmaceuticals, King, PA, U.S.A.). Previously reported groups of nondiabetic rats (Wongmekiat & Johns, 2001a, b) and two groups of diabetic rats ($n=7$–9) in the current study were treated with SB209670 at 10 or 30 $\mu g\,kg^{-1}\,min^{-1}$. SB209670 at 10 $\mu g\,kg^{-1}\,min^{-1}$ has been reported in the rats to reverse completely the haemodynamics effects of an infusion of exogenous ET (Douglas *et al*., 1995; Gardiner *et al*., 1995), but the higher dose was also employed to ascertain that the maximal effects were achieved. After the first two clearances were taken, SB209670 was mixed in an aliquot of the maintenance inulin infusion and given continuously for the duration of the experiment. An interval of 20 min was allowed in order for the compound to equilibrate and develop its full effect before two further 15-min clearances were collected to obtain the new baseline. VE was induced and all clearance collections were then carried out as outlined in series 1.

Series 3: Characterisation of endothelin receptor subtype An additional diabetic group ($n=8$) and a previously reported group of nondiabetic rats (Wongmekiat & Johns, 2001a, b) were studied to evaluate the contribution of the ET_A receptor to the renal responses to volume expansion. A similar protocol to that of series 2 was performed with the difference that this group of rats received UK 350,926 (Pfizer Central Research, Sandwich, U.K.), a highly selective antagonist for ET_A receptors (Huang *et al*., 2002) which has a similar potency for ET_A receptors as SB209670. The compound was mixed in the inulin solution and given continuously at the rate of 30 $\mu g\,kg^{-1}\,min^{-1}$.

Analytical methods

Blood glucose concentration was tested using a glucometer (Model 5529, Miles Laboratories Inc., IN, U.S.A.). Urine volume was measured gravimetrically. Glomerular filtration rate (GFR) was calculated from the clearance of inulin, and inulin concentrations were determined using a diphenylamine colorimetric technique (Bojesen, 1952). The sodium contents in urine and plasma were analysed by flame photometry (Model 410 C, Ciba Corning, U.K.).

Statistical analysis

Data are expressed as means ± s.e.m. Differences within groups were tested using the Student's paired *t*-test. Comparison of means between groups was performed by one-way analysis of variance (ANOVA) followed by a Bonferroni/Dunn *post hoc* test. The repeated-measures ANOVA was used to compare the

profiles of the responses during volume expansion period between the groups. Significance was taken at $P<0.05$.

Results

Body weight, kidney weight, and blood glucose

The values of body weights, kidney weights, and blood glucose concentration in all rats used in this study are given in Table 1. All STZ-diabetic rats gained less body weight than the nondiabetic rats (all $P<0.001$), but they demonstrated marked renal hypertrophy as evident by a greater kidney weight as well as kidney weight/body weight ratio (all $P<0.001$). Diabetic rats also had an approximately four-fold higher blood glucose level compared to the nondiabetic rats (all $P<0.001$). There were no significant differences in any of these parameters between the diabetic groups.

Series 1: Renal responses to acute volume expansion The initial values for MAP, heart rate, and RBF were very similar between the nondiabetic and diabetic rats and these values remained relatively stable throughout the VE period. All basal renal excretory parameters from both kidneys of diabetic rats were also not significantly different from those observed in the corresponding nondiabetic kidneys (Table 2). The denervated kidneys of both nondiabetic and diabetic rats exhibited significantly higher values of urine flow (UV), absolute ($U_{Na}V$), and fractional (FENa) sodium excretion, but not GFR, than the values recorded from their contralateral innervated kidneys (Table 2).

There was a progressive increase in UV (Figure 1) and $U_{Na}V$ (Figure 2) over the course of VE and similar profiles were seen in both nondiabetic and diabetic rats. However, the magnitude of diuresis and natriuresis was markedly attenuated in diabetic rats compared to the nondiabetic rats. The cumulative urine flow (CuUV) after 40 min VE in diabetic rats was 537 ± 43 and $344\pm24\,\mu l\,min^{-1}\,g$ kidney wt^{-1} in the denervated and innervated kidneys, respectively, which were both less (both $P<0.001$) than those obtained in the nondiabetic rats at $1475\pm57\,\mu l\,min^{-1}\,g$ kidney wt^{-1} in the denervated and $1016\pm47\,\mu l\,min^{-1}\,g$ kidney wt^{-1} in the innervated kidneys (Figure 3). Likewise, cumulative urine sodium excretion ($CuU_{Na}V$) in diabetic rats reached values of 116 ± 10 in the denervated and $74\pm6\,\mu mol\,min^{-1}\,g$ kidney wt^{-1} in the innervated kidneys, while they were significantly higher (both $P<0.001$) at 267 ± 9 and $183\pm10\,\mu mol\,min^{-1}\,g$ kidney wt^{-1} in the denervated and innervated kidneys of nondiabetic rats, respectively (Figure 3).

Series 2: Effects of endothelin antagonist on renal responses to volume expansion In previously reported groups of nondiabetic rats (Wongmekiat & Johns, 2001a, b) infusion of SB209670 at 10 and $30\,\mu g\,kg^{-1}\,min^{-1}$ i.v. had no effect on MAP, heart rate, or RBF in either the basal state or during acute saline volume expansion. However, both doses of SB209670 decreased urine flow and sodium excretion by approximately 45–55% in both the innervated and denervated kidneys at low and high doses of the SB209670 (all $P<0.001$). The CuUV responses to acute saline volume expansion were reduced by 38 and 51% in the denervated and by 43 and 50% in the innervated kidneys at the low and high doses of

Table 1 Body weight, kidney weight, and blood glucose level in all experimental groups

Group	*Body weight (g)*	*Kidney weight (g)*		*Kidney weight/Body weight (×100)*		*Blood glucose*
		Left	*Right*	*Left*	*Right*	*(mmol l^{-1})*
Nondiabetic rats	302 ± 5	1.13 ± 0.03	1.17 ± 0.03	0.38 ± 0.01	0.39 ± 0.01	5.0 ± 0.3
Diabetic rats	236 ± 6*	1.42 ± 0.07*	1.45 ± 0.08*	0.60 ± 0.02*	0.61 ± 0.02*	16.7 ± 0.9*
Diabetic rats + SB209670 ($10\,\mu g\,kg^{-1}\,min^{-1}$)	233 ± 8*	1.40 ± 0.06*	1.40 ± 0.05*	0.60 ± 0.02*	0.61 ± 0.02*	17.8 ± 0.8*
Diabetic rats + SB209670 ($30\,\mu g\,kg^{-1}\,min^{-1}$)	230 ± 5*	1.23 ± 0.05*	1.17 ± 0.06*	0.53 ± 0.01*	0.50 ± 0.01*	17.7 ± 0.7*
Diabetic rats + UK 350,926	247 ± 4*	1.32 ± 0.04*	1.38 ± 0.04*	0.53 ± 0.02*	0.55 ± 0.01*	18.1 ± 0.5*

Values are means ± s.e.m. *$P<0.001$ *vs* nondiabetic rats.

Table 2 Basal renal excretory functions in nondiabetic and diabetic rats

Parameters	*Kidney*	*Nondiabetic rats*	*Diabetic rats*
GFR	Denervated	1.1 ± 0.1	0.9 ± 0.1
($ml\,min^{-1}\,g$ kidney wt^{-1})	Innervated	0.9 ± 0.1	0.8 ± 0.1
UV	Denervated	18.9 ± 2.0*	18.8 ± 2.1*
($\mu l\,min^{-1}\,g$ kidney wt^{-1})	Innervated	9.2 ± 1.6	9.3 ± 0.8
$U_{Na}V$	Denervated	3.4 ± 0.4**	2.6 ± 0.4**
($\mu mol\,min^{-1}\,g$ kidney wt^{-1})	Innervated	1.3 ± 0.3	0.9 ± 0.1
FENa (%)	Denervated	1.9 ± 0.2***	1.8 ± 0.2*
	Innervated	1.0 ± 0.3	0.7 ± 0.1

Values are means ± s.e.m. GFR, glomerular filtration rate; UV, urine flow; $U_{Na}V$, absolute sodium excretion FENa, fractional sodium excretion. *$P<0.01$, **$P<0.001$, ***$P<0.05$, *vs* corresponding innervated kidneys.

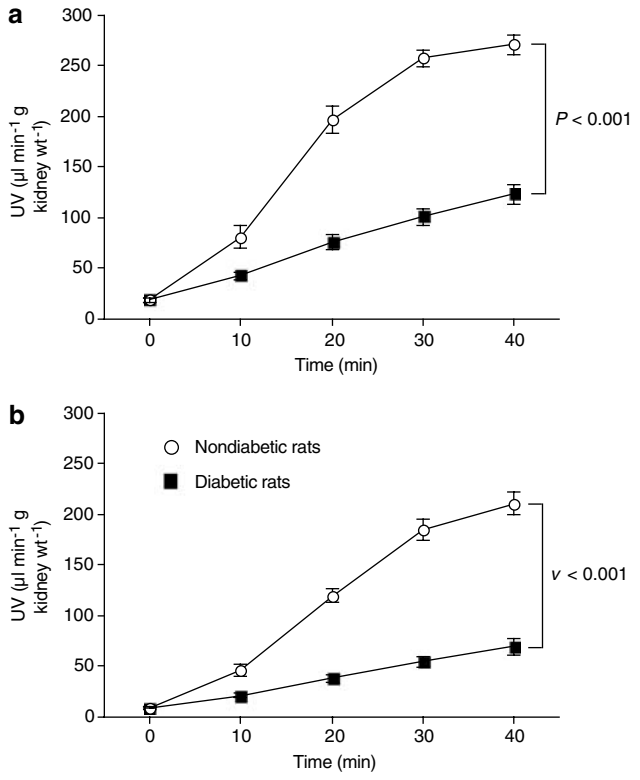

Figure 1 Urine flow (UV) in response to volume expansion in denervated (a) and innervated (b) kidneys of non-diabetic and diabetic rats.

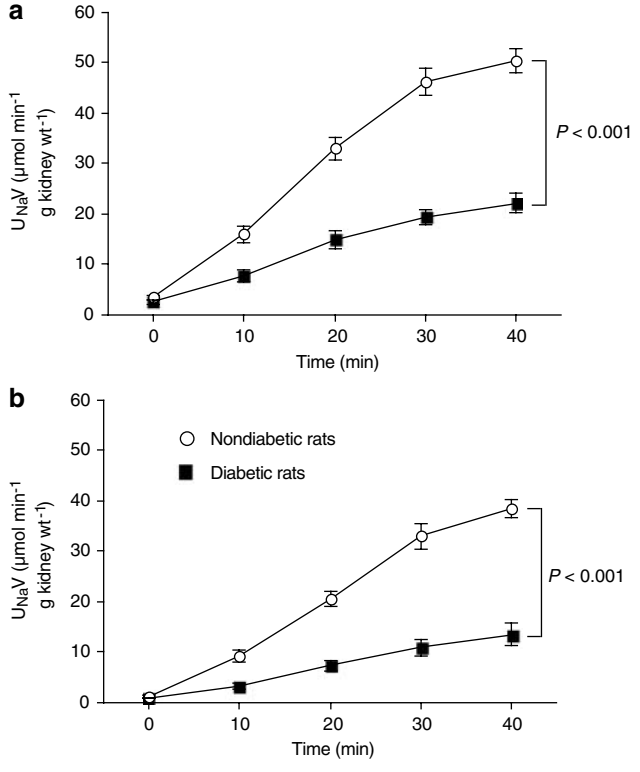

Figure 2 Urine sodium excretion ($U_{Na}V$) in response to volume expansion in denervated (a) and innervated (b) kidneys of non-diabetic and diabetic rats.

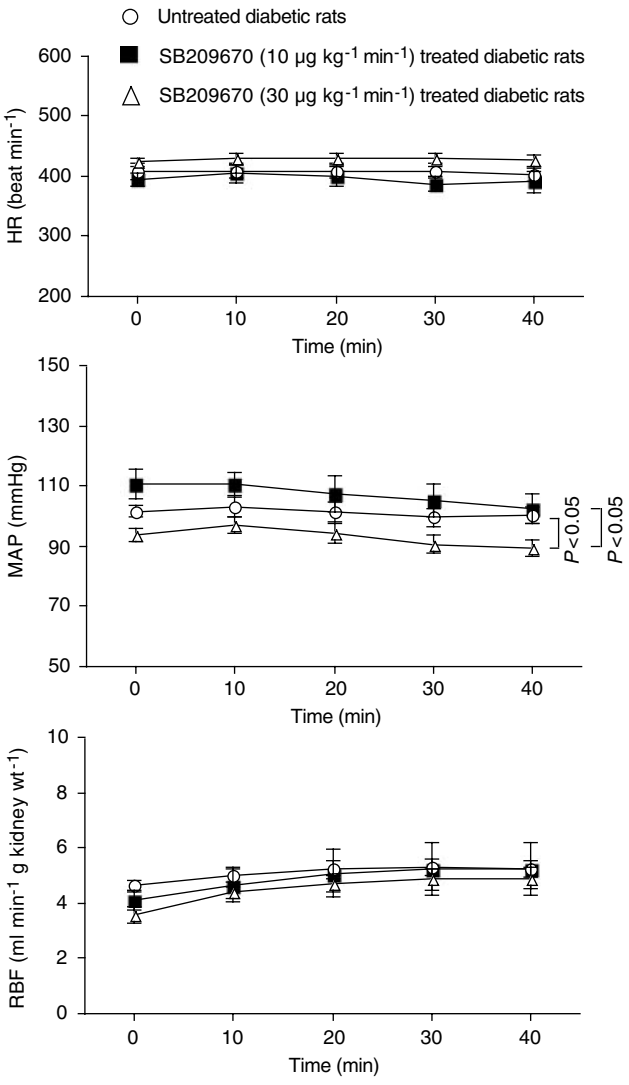

Figure 3 Effects of volume expansion on heart rate (HR), mean arterial blood pressure (MAP), and renal blood flow (RBF) in intrented, 10 µg kg^{-1} min^{-1} SB209670 treated, and 30 µg kg^{-1} min^{-1} SB209670-treated diabetic rats.

SB209670, respectively (all $P<0.001$). At the same time, CuNaV was decreased by 38 and 40% in the denervated and 45 and 41% in the innervated kidneys (all $P<0.001$), respectively, with the low and high doses of SB209670.

Administration of SB209670 at 10 μg kg^{-1} min^{-1} in the diabetic rats had no significant effect on basal heart rate, MAP, RBF or any of the renal excretory variables (Table 3). However, in these diabetic rats, the higher dose of this compound (30 μg kg^{-1} min^{-1}) caused a significant ($P<0.001$) decrease in MAP by some 12%, while it had no influence on heart rate and RBF (Table 3).

As seen in the diabetic rats (Table 3), SB209670 at 30 μg kg^{-1} min^{-1} significantly reduced all basal renal excretory functions. In the denervated kidneys of these diabetic rats, GFR fell slightly (by 17%), whereas UV, $U_{Na}V$, and FENa decreased significantly by 35, 45, and 36%, respectively (all $P<0.05$). A greater decrement in GFR (46%), UV (61%), and $U_{Na}V$ (80%) was observed in the innervated kidneys of these diabetic rats (all $P<0.05$). FENa in the innervated kidneys

Table 3 Effect of SB209670 on basal systemic, renal haemodynamics, and excretory functions in diabetic rats

Parameters	*Kidney*	*SB209670 (10 μg kg^{-1} min^{-1})*		*SB209670 (10 μg kg^{-1} min^{-1})*	
		Before infusion	*After infusion*	*Before infusion*	*After infusion*
MAP (mm Hg)		112±3	110±4	104±1	93±2***
HR (beat min^{-1})		389±13	395±10	413±10	423±7
RBF (ml min^{-1} g kidney wt^{-1})		4.0±0.2	4.1±0.3	3.7±0.4	3.5±0.3
GFR (ml min^{-1} g kidney wt^{-1})	Denervated	1.0±0.1	1.0±0.1	1.2±0.1	1.0±0.1
	Innervated	0.8±0.1	0.7±0.1††	1.1±0.1	0.6±0.1*††
UV (μl min^{-1} g kidney wt^{-1})	Denervated	17.1±2.5	19.2±2.8	39.1±9.0	25.3±4.9*
	Innervated	9.5±1.3†	7.7±1.1††	20.1±6.8†	7.9±2.0*††
$U_{Na}V$ (μmol min^{-1} g kidney wt^{-1})	Denervated	3.2±0.5	3.4±0.6	6.4±1.3	3.5±0.6*
	Innervated	1.6±0.2†	1.1±0.1††	2.5±0.7†	0.5±0.1*††
FENa (%)	Denervated	2.2±0.5	2.4±0.6	3.4±0.6	2.2±0.3*
	Innervated	1.2±0.2	1.0±0.1††	1.4±0.2†	1.0±0.4

Values are means±s.e.m. MAP, mean arterial pressure; HR, heart rate; RBF, renal blood flow; GFR, glomerular filtration rate, Uv, Urine flow, $U_{Na}V$, absolute sodium excretion; FENa, fractional sodium excretion. *$P<0.05$, **$P<0.01$, ***$P<0.001$ before *vs* after infusion. †$P<0.05$, ††$P<0.01$, denervated *vs* innervated kidneys.

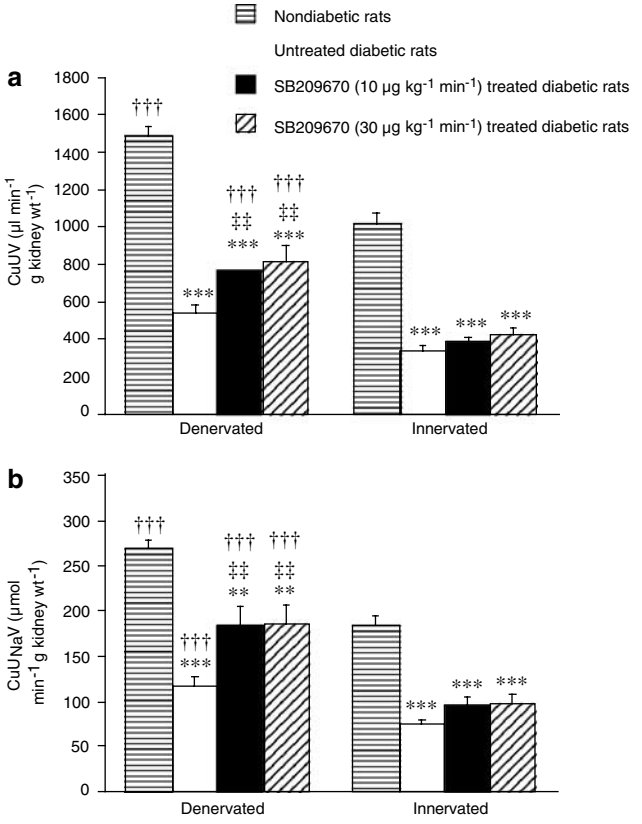

Figure 4 Cumulative urine flow (CuUV) and sodium excretion ($CuU_{Na}V$) after 40 min volume expansion in nondiabetic, untreated diabetic, and SB209670-treated diabetic rats. *$P<0.05$, **$P<0.01$, ***$P<0.001$ *vs* nondiabetic rats. ‡$P<0.05$, ‡‡$P<0.01$, †$P<0.05$, ††$P<0.01$, †††$P<0.001$ *vs* corresponding innervated kidneys.

also showed a tendency to decrease, but the difference did not reach statistical significance. It should be noted that the renal excretory parameters were higher in the denervated than in the contralateral innervated kidneys of these diabetic rats both before (all $P<0.05$) and after (all $P<0.01$) SB209670 infusion.

VE did not affect heart rate, MAP, or RBF in any groups of SB209670-treated diabetic animals (Figure 3) and there were no significant differences in the profiles of heart rate and RBF between the experimental groups throughout the VE period. A significantly lower MAP was observed in the high-dose SB209670 treated group compared to those in the low-dose group and the untreated diabetic group (both $P<0.05$) from the initiation of saline loading through to the end of the experiment (Figure 3).

It was apparent that SB209670, neither the low nor high dose, failed to change the magnitude of CuUV or $CuU_{Na}V$ responses to VE in the innervated kidneys of diabetic rats (Figure 4). CuUV after 40 min VE in both SB209670-treated diabetic groups remained significantly lower than that of the nondiabetic rats by 58–63% (both $P<0.001$), as was $CuU_{Na}V$ which were both lower (both $P<0.01$) by 48% (Figure 4). However, SB209670 was able to enhance the urine flow and sodium excretion responses to saline expansion in the denervated kidneys. CuUV and $CuU_{Na}V$ after VE in the low-dose SB209670-treated diabetic group were greater than those of the untreated diabetic rats by 30 and 37% (both $P<0.01$), respectively (Figure 4). Increasing the dose of SB209670 did not cause any further increase in CuUV or $CuU_{Na}V$ compared to those achieved with the low dose. As shown in Figure 4, the values of CuUV and $CuU_{Na}V$ in the denervated kidneys of both SB209670-treated diabetic groups remained significantly less than those of the nondiabetic rats ($P<0.001$ for CuUV and $P<0.01$ for $CuU_{Na}V$).

Series 3: Characterisation of endothelin receptor subtype In previously reported data in nondiabetic rats, infusion of the ET_A antagonist decreased MAP by some 5 mmHg but depressed basal fluid excretion to the same degree as with the nonselective ET antagonist (Wongmekiat & Johns, 2001a, b) and the excretory responses to acute saline volume expansion, expressed as CuUV and CuNaV, were reduced by 43 and 45%, and 39 and 42% in denervated and innervated kidneys, respectively. Infusion of U.K. 350,926 in STZ-diabetic rats produced a slight, but significant ($P<0.001$), decrease in basal MAP of 12%, without affecting heart rate or RBF (Table 4). These changes in haemodynamics were similar to those of diabetic rats treated with the same dose of SB209670. The initial values for UV and $U_{Na}V$ in the denervated kidneys were significantly higher than those in the contralateral innervated kidneys and remained higher after U.K. 350,926 infusion (both $P<0.05$). However, GFR and FENa were comparable

Table 4 Effect of U.K. 350,926 on basal systemic, renal haemodynamics, and excretory functions in diabetic rats

Parameters	*Kidney*	*UK 350,926*	
		Before infusion	*After infusion*
MAP (mm Hg)		105±1	92±2*
HR (beat min^{-1})		426±12	420±12
RBF (ml min^{-1} g kidney wt^{-1})		4.0±0.1	4.3±0.4
GFR (ml min^{-1} g kidney wt^{-1})	Denervated	1.4±0.3	1.0±0.2**
	Innervated	1.1±0.1	0.7±0.1***
UV (μl min^{-1} g kidney wt^{-1})	Denervated	29.4±3.0	18.1±1.7**
	Innervated	17.3±2.0†	10.7±1.0***†
$U_{Na}V$ (μmol min^{-1} g kidney wt^{-1})	Denervated	5.0±0.9	2.3±0.3**
	Innervated	2.7±0.3†	1.2±0.2***†
FENa (%)	Denervated	2.1±0.7	1.2±0.2
	Innervated	1.4±0.2	0.9±0.1

Values are means±s.e.m. MAP, mean arterial pressure; HR, heart rate; RBF, renal blood flow; GFR, glomerular filtration rate; UV, urine flow; $U_{Na}V$, absolute sodium excretion; FENa, fractional sodium excretion. *$P<0.001$, **$P<0.05$, ***$P<0.01$ before *vs* after infusion. †$P<0.05$ denervated *vs* innervated kidneys.

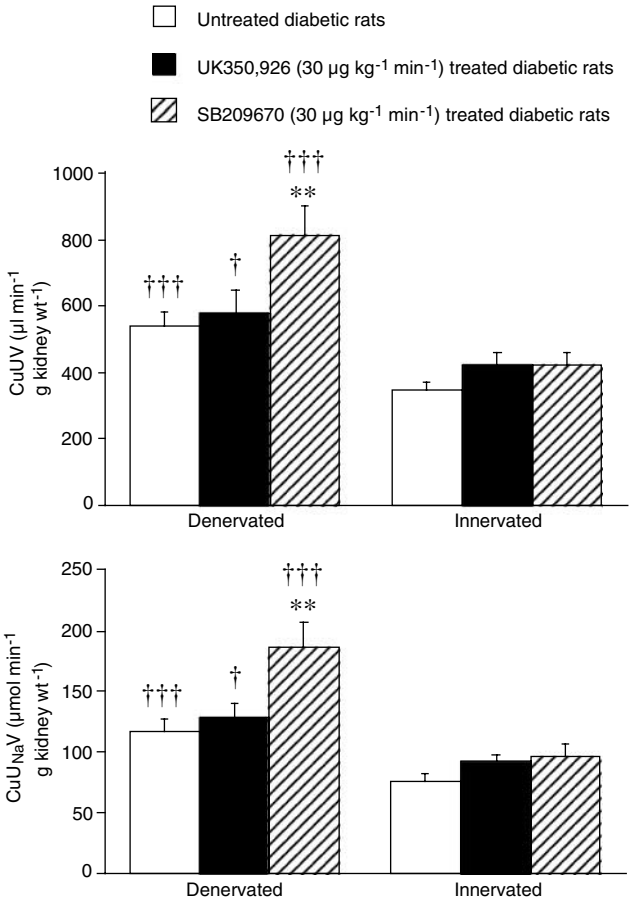

Figure 5 Effects of UK 350,926 and SB209670 on cumulative urine flow (CuUV) and sodium excretion (Cu) in diabetic rats. *$P<0.05$, **$P<0.01$ *vs* untreated diabetic rats. †$P<0.05$, ††$P<0.01$, †††$P<0.001$ *vs* corresp.

between the two kidneys both before and after the infusion of U.K. 350,926. As shown in Table 4, GFR, UV, and $U_{Na}V$ were all reduced significantly after U.K. 350,926 administration by 29, 39, and 54% in the denervated kidneys (all $P<0.05$) and by 36, 38, and 56% in the innervated kidneys, respectively (all $P<0.01$). FENa also showed a tendency to decrease in both kidneys, but this reduction did not reach statistical significance.

MAP, heart rate, and RBF over the period of VE in the group receiving U.K. 350,926 were comparable to those of diabetic rats treated with the same dose of SB209670. However, CuUV and $CuU_{Na}V$ responses to VE in the denervated kidneys, but not innervated kidneys, of U.K. 350,926-treated rats were shown to be significantly less than those obtained when both endothelin receptor subtypes were blocked by SB209670 (Figure 5). At the end of VE, CuUV and $CuU_{Na}V$ in the denervated kidneys of U.K. 350,926-treated group were lower (both $P<0.05$) than those of the SB209670-treated group by 32 and 29%, respectively. Despite the lower ($P<0.05$) profile of MAP during VE in the U.K. 350,926-treated group, the magnitude and pattern of diuretic and natriuretic responses to VE in this group were virtually the same as those seen in untreated diabetic rats (Figure 5).

Discussion

Infusion of a nonselective ET_A/ET_B antagonist, SB209670 at 10 $\mu g\,kg^{-1}\,min^{-1}$ in the diabetic rats had minimal effects on basal systemic, renal haemodynamics, and renal excretory function, while the reductions in these parameters were apparent when a higher dose of SB209670 (30 $\mu g\,kg^{-1}\,min^{-1}$) was employed. We have previously shown that SB209670 even at 10 $\mu g\,kg^{-1}\,min^{-1}$ produced a fall in these parameters of some 40–50% in nondiabetic rats (Wongmekiat & Johns, 2001b). These data would be compatible with the view that the ET system was overactive in the diabetic condition, which would correspond to earlier reports demonstrating an elevated circulating plasma level as well as urinary ET-1 excretion in both diabetic patients and animals (Morabito *et al*., 1994; Hocher *et al*., 1998; Makino & Kamata, 1998).

Basal renal excretory functions from both kidneys of diabetic rats were reduced after infusion of SB209670 at 30 $\mu g\,kg^{-1}\,min^{-1}$, but these reductions may be related to the decrease in systemic arterial pressure. However, a potential direct tubular effect of ET could not be discounted since MAP fell only slightly and it was well within the autoregulatory range. A small, albeit not significant, increase in heart rate, together with an unchanged RBF, accompanying the fall in

MAP implied that autoregulation has been called into play. Furthermore, the observation that UV, $U_{Na}V$, and FENa decreased to a greater extent than GFR, implied that the reduction in filtered load may not be totally responsible for the overall reduction in water and sodium excretion. Of interest is the finding that the magnitude of decrement in all these renal excretory parameters in the innervated kidney was almost twice as great as those of the denervated kidney, suggesting that sympathetic tone to the kidney might be augmented after blockade of the action of ET. This contrasts with the situation in the nondiabetic rats (Wongmekiat & Johns, 2001a, b) in which the reduction was equivalent in both innervated and denervated kidneys. One simplistic explanation would be that in the diabetic animals the sympathoexcitation may be the consequence of baroreflex activiation, but a direct interaction between ET and the sympathetic nervous system cannot be excluded since there are reports of changed activity in sympathetic nerves after ET administration, although the direction of the interaction remains uncertain (Wong-Dusting *et al*., 1990; Lerman *et al*., 1991; Takagi *et al*., 1991).

The present data demonstrated that blocking the action of ET with SB209670 significantly ameliorated the blunted diuretic and natriuretic responses to VE in the denervated diabetic kidney. This is in contrast to the observations in the nondiabetic state where the SB209670 blunted the excretory responses to saline volume expansion (Wongmekiat & Johns, 2001a, b). The present finding would indicate that ET-1 was one of the contributory factors in causing the blunted volume reflex observed in diabetes mellitus. A further point is that the enhanced ability to excrete a saline load in the diabetic rats in the presence of SB209670 was undoubtedly mediated by attenuating a tubular action of ET since MAP and RBF over the VE period were not different between the untreated and low-dose SB209670-treated diabetic animals. More convincing evidence was the finding that this enhanced effect occurred even though the MAP was lower as was apparent in the high-dose SB209670-treated group. In fact, the lower MAP present in the high-dose SB209670-treated group may explain, in part, why there was no dose-dependent improvement in the blunted volume reflex. The incidental observation that the ability of SB209670 to enhance fluid mobilization was not observed in the innervated kidney, implied that the impact of the blockade ET receptors was overridden by a tonic influence of the renal nerves. Again, it is likely that there are two components to this interaction; the higher basal renal sympathetic nerve activity as a consequence of baroreflex function and the direct action of ET on sympathetic activity.

The question then arises as to which receptor subtype may be responsible for the renal excretory changes observed after infusion of a nonselective ET_A/ET_B antagonist. Additional studies demonstrated that selective blockade of ET_A receptors with U.K. 350,926 produced alterations in basal MAP which was comparable to that recorded when the nonselective ET_A/ET_B antagonist was given at the same dose. This observation suggested that endogenous ET played a role in the maintenance of resting blood pressure in the diabetic condition that appeared to be mediated by ET_A receptors. With respect to the renal excretory function, the data obtained under basal conditions do not allow a clear conclusion to be drawn. This is because blockage of ET_A and ET_B or ET_B receptors alone caused significant reductions in blood pressure in the diabetic but not in the nondiabetic rats which of itself would have had a direct effect to decrease fluid excretion. It is then difficult from these studies to separate a direct action of pressure from that of a tubular action of endothelin from causing the antinatriuresis. However, it was clear that during volume excretion a role for the ET_B receptors became evident.

The systemic and renal haemodynamic responses to VE in the U.K. 350,926-treated diabetic rats were also similar to those observed in the SB209670-treated animals. Nevertheless, the diuresis and natriuresis induced by volume loading in U.K. 350,926-treated rats were not different from those achieved in the untreated diabetic rats regardless of whether or not the renal nerves were intact. This is in contrast to the blunted excretory responses in the nondiabetic rats in which ET_A receptors were blocked. The reason for this difference in the action of the ET_A selective antagonist on VE in diabetic versus nondiabetic rats is unclear. It may be indicative of either an inability to decrease production of endothelin in response to increased fluid excretion or to a change in the proportion of renal ET_A *versus* ET_B receptors in the diabetic state. The present data not only lend credence to the potential tubular actions of ET but also to the possible role of blockade of ET_B receptors in mediating the enhanced excretory responses to volume loading in diabetes mellitus.

Interestingly, the results presented herein clearly demonstrated a disparity in the role of ET in nondiabetic and diabetic conditions. We have previously shown that blocking the actions of ET using both nonselective and ET_A selective receptor antagonists in nondiabetic rats caused a depressed ability to excrete a saline load (Wongmekiat & Johns, 2001b). However, it appeared in the current study that similar manipulation enhanced this ability in diabetic rats. This finding would support the notion that ET may exert its effects under physiological and pathological conditions in different ways and cause opposite responses. This hypothesis was based on previous reports that the effects of ET, even in the same organ, depended on its mode of action (Rubanyi & Polokoff, 1994). Apparently, ET appears to function predominantly as a local rather than a circulating hormone in physiological circumstances; however, as its production is increased, the ET overflow may evoke profound systemic effects, which causes a different response (Kohan, 1997). In addition, several investigators have postulated that low doses of ET elicited a natriuresis, while high doses caused an antinatriuresis (Sandgaard & Bie, 1996). These observations could apply to the present study where the renal ET system appears overstimulated in the diabetic state. Indeed, an alteration in the density, proportion, distribution, binding affinity, and actions of ET receptor subtypes have all been reported under some pathophysiological conditions (Koide *et al*., 1995; Gellai *et al*., 1994; Qiu *et al*., 1995). This would be consistent with the present findings, which showed that the effect of ET on renal excretory responses to VE in diabetic rats appeared to be mediated by ET_B receptors, while it was ET_A receptors that displayed this effect in nondiabetic rats (Wongmekiat & Johns, 2001b).

Overall, the results of this study are consistent with the view that diabetes mellitus is associated with an overstimulation of the ET system. It also provided evidence for the role of ET in blunted reflex renal responses to VE observed in diabetes mellitus. The data show that ET contributes to this reflex abnormality with the evidence suggesting that the action is mediated via an ET_B receptor subtype, but its impact is

obscured by the tonic influence of the renal sympathetic nerves. This view is derived from the differential effect of a nonselective ET_A/ET_B and a selective ET_A receptor antagonist. What is now important is to reinforce this view using a selective ET_B antagonist.

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