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Vascular reactivity to angiotensin II in blood-perfused kidneys of hypertensive diabetic rats

Nicole K. Farina [1], Wayne C. Hodgson [*], Robert E. Widdop

Department of Pharmacology, Monash University, Clayton, Victoria 3168, Australia

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Abstract

The present study examined vascular reactivity to angiotensin II in blood-perfused kidneys of diabetic normotensive Wistar-Kyoto (WKY) and diabetic spontaneously hypertensive rats (SHR). In addition, the effect of the angiotensin AT_1 receptor antagonist, CV-11974 (2-ethoxy-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1*H*-benzimidazole-7-carboxylic acid), on angiotensin II responses was examined. Dose-response curves to angiotensin II (0.1–30 μg/kg, i.a.) were obtained in kidneys of control- and diabetic-WKY rats and -SHR rats, either in the absence or presence of CV-11974 (3 μg/kg, i.v.). In all four treatment groups, angiotensin II produced dose-dependent increases in renal perfusion pressure with the order of reactivity: control-SHR > control-WKY = diabetic-SHR > diabetic-WKY. In the presence of CV-11974 (3 μg/kg, i.v.), dose-response curves to angiotensin II were significantly inhibited in kidneys of control-SHR and -WKY rats. However, CV-11974 (3 μg/kg, i.v.) had no significant effect on angiotensin II responses in kidneys of diabetic-SHR or -WKY rats. These results suggest that diabetes in normotensive rats is associated with impaired renal responsiveness to angiotensin II, while hypertension augments renal responsiveness to angiotensin II. However, the combination of diabetes and hypertension has largely offset the opposite effects on angiotensin II responses seen separately. Importantly, the lack of effect of CV-11974 in diabetic rats, with or without hypertension, has been identified. While the reasons for these alterations have yet to be determined, they may involve changes in angiotensin II receptor mechanisms (e.g. density and/or affinity).

Keywords: Diabetes; Hypertension; Angiotensin; Blood perfused; Nephropathy; Kidney; (Rat)

1. Introduction

Diabetic and hypertensive nephropathy are the most common causes of end-stage renal disease (Walker, 1993). These diseases frequently co-exist and together increase the risk and accelerate the progression of nephropathy in clinical (Marre et al., 1993) and experimental (Cooper et al., 1986) diabetes. While it has been estimated that 100% of long-term insulin-dependent diabetics will develop either retinopathy or neuropathy, only a subpopulation (30–40%) will develop nephropathy (Adler, 1993; Marre et al., 1993). Clinical studies have suggested that this subpopulation of patients also have a genetic predisposition to hypertension (Chukwuma, 1992; Krolewski et al., 1988). Therefore, it is possible that hypertension is a pathogenetic factor in the development of diabetic nephropathy (Cooper et al., 1986).

Rats with streptozotocin-induced diabetes, an animal model of insulin-dependent diabetes, display alterations in renal function which are characteristic of clinical diabetic nephropathy (Mogensen and Anderson, 1975). However, experimental models of diabetes mellitus have not generally been associated with significant hypertension (Tomlinson et al., 1992). Therefore, an animal model combining genetic hypertension (spontaneously hypertensive rat (SHR)) and diabetes is considered to be a more accurate model for investigating the role of hypertension as a cause or accelerator of diabetic nephropathy (Cooper et al., 1988).

The ability of the angiotensin-converting enzyme inhibitors and angiotensin AT_1-receptor antagonists to confer renal protection (Cooper et al., 1990; Lewis et al., 1993; Soltis, 1993) has highlighted the importance of angiotensin II activity in the progression of renal disease during both disease states.

* Corresponding author. Tel.: 61 3 9905 4861; fax: 61 3 9905 5851.

[1] Present address: Department of Medicine, Austin Hospital, Heidelberg, Victoria, Australia, 3084.

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Previous studies have examined the biochemical and morphological characteristics of the hypertensive diabetic rat. However, these indices are usually measured after long-term diabetes and/or hypertension (e.g. 16–32 weeks; Cooper et al., 1988, 1990). In contrast, changes in renal vascular reactivity in this model have not been examined. Therefore, the present study examined changes in renal vascular responsiveness to angiotensin II in the SHR 6 weeks after the induction of diabetes. This is, according to previous studies, before overt biochemical or morphological renal changes become evident (Cooper et al., 1986, 1988).

2. Materials and methods

2.1. Induction of diabetes

Male Wistar Kyoto (WKY) and SHR rats weighing 240–340 g (11–13 weeks of age), were anaesthetised with halothane (4%; 2:1 O_2/N_2O) and made diabetic by the administration of streptozotocin (WKY, 60 mg/kg; SHR, 45 mg/kg) via a tail vein. A solution of the diabetogen in citrate buffer (50 mM, pH 4.5) was prepared immediately prior to injection. Age and strain-matched controls received an equivalent volume of citrate buffer. Prior to injection, animal weights and blood glucose levels were recorded. Animals were subsequently housed in treatment pairs for 6 weeks with free access to food and water.

Systemic blood pressure was measured prior to injection, and then 2, 4 and 6 weeks later, by indirect tail-cuff plethysmography in conscious rats.

2.2. Blood-perfused kidneys

After 6 weeks, animals were weighed and anaesthetised with sodium pentobarbitone (60–100 mg/kg, i.p.). A blood sample, from the tail vein, was taken to determine blood glucose levels (Ames Minilab 1). Animals were considered diabetic if their blood glucose concentration was > 17 mM. Control rats exhibited blood glucose levels between 3–10 mM. The blood-perfused kidneys preparation was set up as described previously (Hodgson et al., 1990). A midline incision was made in the cervical region, and the trachea cannulated. The right jugular vein was cannulated for the administration of heparinised saline (500 units/kg). Arterial blood pressure was measured from a cannula in the right carotid artery. An abdominal midline incision was made and the abdominal aorta ligated below and above the kidneys. The aorta was subsequently cannulated between the two ties, and both kidneys perfused with blood from the left carotid artery, using a Masterflex pump (Model 7554-10). Arterial and perfusion pressures were monitored using Gould-Statham pressure transducers (P23) and recorded on a Grass Polygraph (Model 79D). The flow rate of the pump was set so that renal perfusion pressure matched systemic blood pressure and thereafter the flow within the circuit was maintained at this rate for the entire experiment. Angiotensin II was administered via bolus injection directly into the aortic tubing using 25 μl or 50 μl microsyringes (Scientific Glass Engineering, Australia). Changes in perfusion pressure indicated alterations in renal vascular resistance. A rectal thermometer was used to ensure the body temperature of the rat was maintained at approximately 37°C. Where necessary, animals were ventilated with a rodent respiratory pump (50 strokes/min; 1 ml/100 g body weight/stroke; Ugo Basile, Model 7025).

In preliminary experiments, optimal conditions for the experimental protocol were determined in control-WKY rats. Two dose-response curves, separated by at least 30 min, were constructed to angiotensin II. Each dose of angiotensin II was added 5–10 min after the previous response had returned to baseline levels. In conjunction with these time control studies an appropriate concentration of CV-11974 was established. It was found that CV-11974 (1 μg/kg, i.v.), caused a small rightward shift in the dose-response curve to angiotensin II. However, this shift was not significantly different from the effect observed in time control experiments (i.e. consecutive dose-response curves to angiotensin II revealed marked tachyphylaxis). Given these data, and the fact that in additional experiments CV-11974 (10 μg/kg, i.v.) abolished the renal vasoconstrictor effects of angiotensin II in kidneys of control and diabetic rats, it was decided to modify the experimental protocol so that only one dose-response curve to angiotensin II was constructed in each animal. In addition, an intermediate dose (3 μg/kg, i.v.) of CV-11974 was chosen for further experiments. This was given prior to the commencement of a dose-response curve to angiotensin II.

2.3. Drugs

The following drugs were used: angiotensin II (American Peptide Co.), CV-11974 (2-ethoxy-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1*H*-benzimidazole-7-carboxylic acid, Takeda Chemical Industries), streptozotocin (Sigma).

Streptozotocin was dissolved in citrate buffer (50 mM, pH 4.5). Angiotensin II was dissolved and diluted in 0.9% saline and kept on ice for the duration of the experiment. CV-11974 was initially dissolved in saline and 1 M Na_2CO_3 (9:1) and further diluted in saline.

2.4. Statistics

Angiotensin II-evoked increases in renal perfusion pressure were compared across treatment groups, or following CV-11974 pretreatment, by two-way analysis of variance (ANOVA) with repeated measures, using a commercially available statistical package (CLR ANOVA). In antagonist studies, where ANOVA revealed a significant effect of CV-11974, the magnitude of the rightward shift in the

dose-response curves to angiotensin II, in the absence or presence of CV-11974, was calculated by performing potency ratios according to published methods (Lentner, 1982) using computer-assisted regression analysis. Other indices (e.g. changes in blood glucose, body weight and systolic blood pressure) were analysed by two-way ANOVA. In all cases, statistical significance is indicated by $P < 0.05$.

3. Results

3.1. Resting variables

As indicated in Table 1, 6 weeks after streptozotocin treatment, WKY and SHR rats displayed significantly increased blood glucose levels, compared to their pretreatment values, and compared to their non-diabetic counterparts ($P < 0.05$, ANOVA). Both diabetic groups displayed similar degrees of hyperglycaemia. Blood glucose levels of control-WKY and -SHR remained within the normal range (i.e. 3–10 mM) over the 6 week period. Streptozotocin-induced diabetes was also associated with a significant reduction in body weight. After 6 weeks, body weights of diabetic-WKY and -SHR rats were significantly lower than their corresponding pre-injection weights ($P < 0.05$, ANOVA, Table 1). Body weight of control-WKY and -SHR significantly increased over the same time period ($P < 0.05$, ANOVA, Table 1).

Throughout the 6 week period, systolic blood pressure (measured indirectly) of control- and diabetic-SHR was significantly higher than that of control- and diabetic-WKY, respectively ($P < 0.05$, ANOVA, Fig. 1). While systolic blood pressure of the WKY groups did not vary considerably over the 6 weeks, there was a significant fall in systolic blood pressure of diabetic-SHR. This resulted in a significant difference in blood pressure between control- and diabetic-SHR at week 6 ($P < 0.05$, ANOVA, Fig. 1).

Kidney weights of diabetic rats were significantly increased compared to those of their controls ($P < 0.05$, ANOVA, Table 1).

Table 2 shows the mean renal flow rate through the circuit in order for the perfusion pressure to match systemic blood pressure. The renal perfusion pressure of control-SHR was significantly increased compared to the other groups. However, the perfusion pressure obtained in kidneys of diabetic-SHR was significantly reduced compared to the perfusion pressures obtained in control- and diabetic-WKY rats ($P < 0.05$, ANOVA, Table 2).

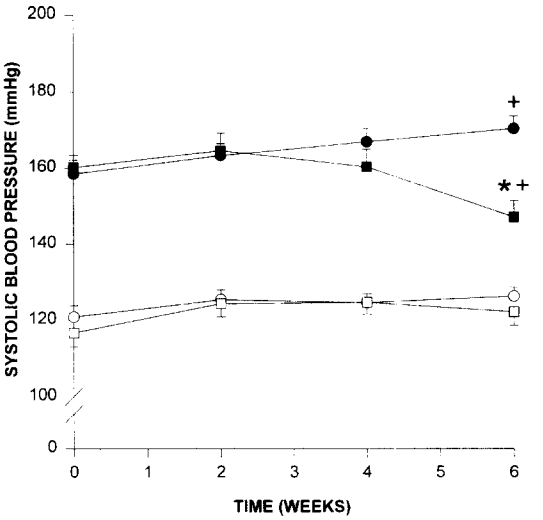

Fig. 1. Indirect systolic blood pressure of control-WKY (open circles, $n = 24$), diabetic-WKY (open squares, $n = 25$), control-SHR (closed circles, $n = 26$) and diabetic-SHR (closed squares, $n = 23$) prior (week 0) and 2, 4, and 6 weeks after injection. * $P < 0.05$, significantly different from initial value (week 0) in same group. + $P < 0.05$, significantly different from final value (week 6) in corresponding WKY group.

3.2. Constrictor responses to angiotensin II

Angiotensin II (0.1–30 μg/kg, i.a.) produced dose-dependent increases in perfusion pressure in kidneys of rats from all 4 experimental groups. In both diabetic groups, the responses to angiotensin II were significantly attenuated compared with their respective control group. The maximum response to angiotensin II was greatest in control-SHR, with the following rank order of reactivity being obtained: control-SHR > control-WKY = diabetic-SHR > diabetic-WKY ($P < 0.05$, ANOVA, Fig. 2). There was a minimal delayed effect of angiotensin II in the systemic circulation following these local injections.

3.3. AT_1-receptor antagonist studies

Dose-response curves to angiotensin II in the presence of CV-11974 (3 μg/kg, i.v.) were obtained in kidneys of

Table 1
Body weight, blood glucose level and kidney weight of control (c) and diabetic (d) normotensive (WKY) and hypertensive (SHR) rats ($n = 16$–31)

	Body weight (g)		Blood glucose level (mM)		Kidney weight (g)	
	Initial	Final	Initial	Final	Left	Right
c-WKY	297 ± 4	351 ± 5 [a]	7.7 ± 0.3	9.2 ± 0.5	1.32 ± 0.03	1.32 ± 0.03
d-WKY	299 ± 5	249 ± 5 [a,b]	8.4 ± 0.5	27.7 ± 0.9 [a,b]	1.58 ± 0.04 [b]	1.53 ± 0.04 [b]
c-SHR	269 ± 4	327 ± 6 [a]	7.9 ± 0.5	7.3 ± 0.3	1.22 ± 0.03	1.23 ± 0.04
d-SHR	287 ± 4	217 ± 8 [a,b]	8.6 ± 0.4	27.8 ± 1.4 [a,b]	1.32 ± 0.05 [b]	1.27 ± 0.06

Initial measurements were made at the time of streptozotocin or vehicle injection, and final measurements made 6 weeks later. [a] Significantly different from initial value in same treatment group, $P < 0.05$ ANOVA. [b] Significantly different from corresponding control, $P < 0.05$ ANOVA.

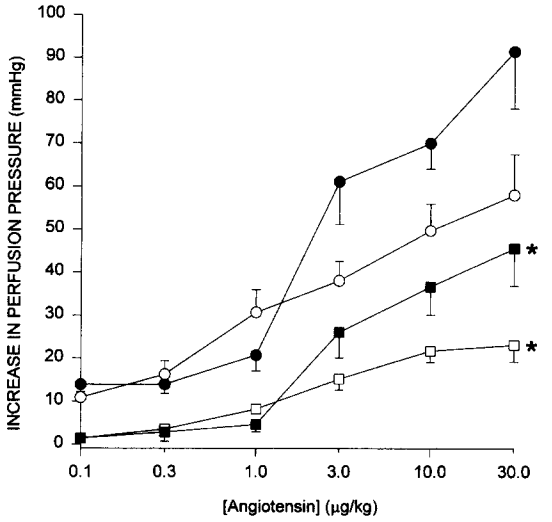

Fig. 2. Discrete dose-response curves to angiotensin II in blood-perfused kidneys of control-WKY (open circles, $n = 11–15$), diabetic-WKY (open squares, $n = 13–15$), control-SHR (closed circles, $n = 8$) and diabetic-SHR (closed squares, $n = 7–9$). * $P < 0.05$, significantly different from corresponding control group, ANOVA.

Table 2
Basal perfusion pressure and flow rate of control (c) and diabetic (d) normotensive (WKY) and hypertensive (SHR) rats ($n = 16–31$)

	Basal perfusion pressure (mm Hg)	Flow rate (ml/min)
c-WKY	105 ± 11	3.8 ± 0.3
d-WKY	87 ± 10	3.4 ± 0.2
c-SHR	137 ± 11	4.1 ± 0.3
d-SHR	78 ± 18 [a]	2.7 ± 0.3

[a] Significantly different from corresponding control, $P < 0.05$ ANOVA.

control- and diabetic-SHR and WKY rats. In control-WKY (Fig. 3a), a significant difference was observed between dose-response curves obtained in the absence or presence of CV-11974 (3 μg/kg, i.v.) when analysed by ANOVA ($P < 0.05$). In addition, the magnitude of the rightward shift was calculated to be 4.3 (1.6, 18.6, 95% c.l.; 88 d.f.). In contrast, in diabetic-WKY, CV-11974 (3 μg/kg, i.v.) had no significant effect on angiotensin II responses (Fig. 3b). In control-SHR there was a significant difference between dose-response curves obtained in the absence or presence of CV-11974 ($P < 0.05$, ANOVA; Fig. 4a). The

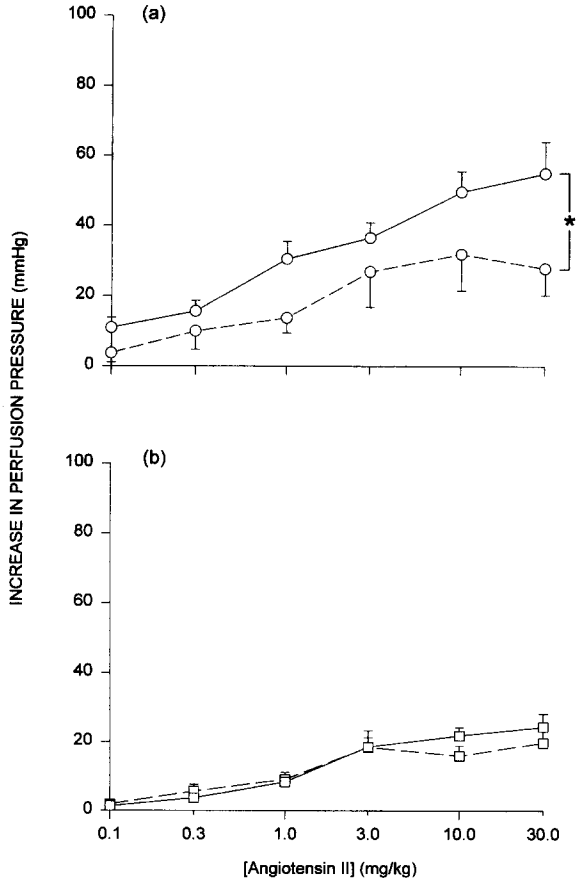

Fig. 3. Discrete dose-response curves to angiotensin II in blood-perfused kidneys of (a) control-WKY rats in the absence ($n = 11–15$, solid line) or presence ($n = 8$, dotted line) of CV-11974 (3 μg/kg, i.v.) and (b) diabetic-WKY rats in the absence ($n = 13–15$, solid line) or presence ($n = 8$, dotted line) of CV-11974 (3 μg/kg, i.v.). * $P < 0.05$, significant difference between dose-response curves, ANOVA.

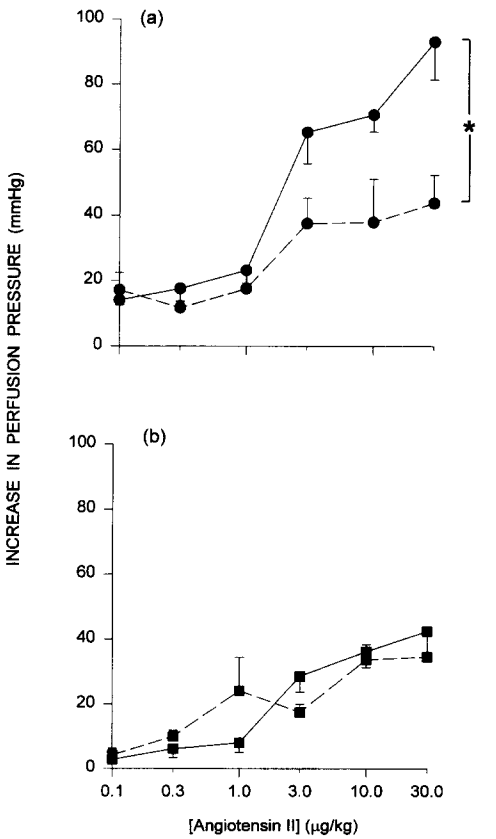

Fig. 4. Discrete dose-response curves to angiotensin II in blood-perfused kidneys of (a) control-SHR rats in the absence ($n = 8$, solid line) or presence ($n = 6$, dotted line) of CV-11974 (3 μg/kg, i.v.) and (b) diabetic-SHR rats in the absence ($n = 7–9$, solid line) or presence ($n = 6$, dotted line) of CV-11974 (3 μg/kg, i.v.). * $P < 0.05$, significant difference between dose-response curves, ANOVA.

magnitude of the rightward shift was calculated to be 3.0 (1.3, 8.5, 95% c.l; 47 d.f.). However, in kidneys of diabetic-SHR, CV-11974 (3 μg/kg, i.v.) had no significant effect on angiotensin II responses (Fig. 4b). CV-11974 had no significant effect on resting perfusion pressure in any of the 4 experimental groups (data not shown).

4. Discussion

The hypertensive diabetic model displays many of the classical features of clinical diabetes, including progressive weight loss, an elevation of blood glucose levels and diabetic nephropathy (Cooper et al., 1986, 1988). Weight loss and hyperglycaemia were clearly evident in the present investigation. The results of the current study also support the previous finding that SHR exhibit a greater susceptibility to the diabetogenic effect of streptozotocin (Somani et al., 1979; Cooper et al., 1988). This was demonstrated by different doses of streptozotocin, 45 and 60 mg/kg (i.v.), producing similar degrees of hyperglycaemia (after 6 weeks) in SHR and WKY rats, respectively.

Control- and diabetic-SHR remained hypertensive, compared with their respective WKY groups, over the 6-week period. However, diabetes of 6 weeks duration, in the SHR, was associated with a decrease in systolic blood pressure. This finding is consistent with earlier work by Yamamoto (1988) and Kam et al. (1994) which showed that diabetic-SHR rats (4–20 weeks duration) exhibited a significant decrease in systolic blood pressure (measured either directly or indirectly), compared to non-diabetic SHR, although the reason for this decrease in systolic blood pressure remains to be elucidated.

Diabetic-SHR have previously been studied for functional and structural changes associated with diabetic nephropathy. Indeed, it has been reported that the hypertensive diabetic rat exhibits mesangial expansion, glomerular basement membrane thickening and an earlier rise in urinary albumin excretion (Cooper et al., 1988), compared to the normotensive diabetic-WKY rat, all of which indicate the accelerated progression of nephropathy. These changes have been documented in SHR with diabetes ranging from 8 to 32 weeks duration (Cooper et al., 1988). However, renal vascular reactivity has not been examined in this model. Therefore the present study determined if vascular changes occur at an earlier time. If so, these may contribute to the structural and functional alterations previously observed.

The present finding that normotensive diabetic rats exhibit attenuated angiotensin II responsiveness, compared to controls, supports earlier work by Sarubbi et al. (1989). These workers demonstrated that angiotensin II-induced increases in renal perfusion pressure, in the isolated Krebs-perfused kidney preparation, were significantly impaired at 2 and 8–12 weeks of diabetes. In addition to animal studies, attenuated renal and systemic responsiveness to angiotensin II has been documented in insulin-dependent diabetic patients (Fioretto et al., 1991). Ballermann et al. (1984) examined glomerular angiotensin II receptor density in early (3–4 weeks) untreated streptozotocin-induced diabetic rats and suggested that the reactivity changes observed may be due to down-regulation of angiotensin II receptor density within the glomerulus. In contrast, Wilkes (1987) demonstrated that although glomerular angiotensin II receptor density was reduced 24 h after the onset of diabetes, a progressive return of receptor density was observed over the following 60 day period. Although plasma renin activity and plasma angiotensin II concentrations were not altered over the 60 days, plasma aldosterone concentrations increased 2-fold. It was concluded that the return of angiotensin II binding sites was due to elevations in plasma aldosterone (Wilkes, 1987). Despite discrepancies between studies, in general, diabetes has been associated with a reduction in angiotensin II receptor numbers in the glomerulus (Ballermann et al., 1984; Brown and Sernia, 1993). This suggests that the diabetic state regulates angiotensin II receptors and that this regulation may be important in the renal vascular changes which characterise diabetic nephropathy. These data are interesting in light of the renal haemodynamic changes which are known to occur during diabetes (Kiff et al., 1991a,b; Tomlinson et al., 1992). In particular, diabetes has been suggested to be associated with renal afferent vasodilation resulting in direct transmission of systemic blood pressure to the exposed kidneys (Cooper et al., 1990). Therefore, renal arteriolar vasodilation may contribute to the pathological changes in kidney structure and function. The fact that the present study reported a decrease in renal responsiveness to angiotensin II during diabetes is consistent with this idea.

These data contrast with those obtained in the hypertensive rats where reactivity to the pressor effects of angiotensin II was found to be increased. This supports the earlier work of Tuncer and Vanhoutte (1991) who reported that responses to angiotensin II were augmented in the isolated Tyrode's-perfused kidney of SHR. Indeed, angiotensin II receptor density has been reported to be higher in glomeruli from SHR compared with WKY rats (Makarious et al., 1993), while diabetes per se has been associated with a down-regulation of glomerular angiotensin II receptors (Ballermann et al., 1984; Brown and Sernia, 1993).

Although renal vascular responsiveness to angiotensin II has been previously examined in rats with either hypertension or diabetes, the present study is the first to document renal vascular responsiveness to angiotensin II in a model combining these disease states. Interestingly, absolute reactivity to angiotensin II in these rats was between that obtained in kidneys of rats with either hypertension or diabetes, alone. As previously mentioned, angiotensin II receptor density has been reported to be higher in glomeruli

from SHR compared with WKY rats (Makarious et al., 1993), while diabetes per se has been associated with a down-regulation of glomerular angiotensin II receptors (Ballermann et al., 1984; Brown and Sernia, 1993). Therefore, it is possible that each of these described changes has offset the other.

One complicating factor in the interpretation of these data was the fact that resting renal perfusion pressure was lower in the diabetic-SHR compared with control-SHR. This could represent a failure of autoregulatory mechanisms in that a lower flow rate to the kidneys of diabetic-SHR was required in order to match renal and systemic pressures, although the reason for the fall in systemic pressure in this group only is not known. In this context, Mauer et al. (1992) have shown that renal blood flow was better maintained at low perfusion pressures in anaesthetised diabetic rats than in control rats. However, direct comparisons with diabetic-SHR in the present study cannot be made. In the present study, if data were normalised to account for differences in basal states of perfusion pressure, renal angiotensin II reactivity was similar in control-SHR and diabetic-SHR (although absolute increases in renal perfusion pressure evoked by angiotensin II were larger in the former group). By contrast, the presence of diabetes in WKY rats impaired angiotensin II reactivity (in both normalised and absolute terms). In any case, renal vasoconstrictor responses to angiotensin II were well preserved in diabetic-SHR relative to their normotensive counterpart. In an isolated perfused mesenteric preparation, it was reported that methoxamine-induced vasoconstriction was similarly less efficacious in diabetic-WKY compared with diabetic-SHR, although the rank order of absolute reactivity to methoxamine in the same 4 experimental groups was different to that seen in the present study (Hendriks et al., 1993), which is suggestive of agonist-specific changes in vasoconstrictor reactivity. Further evidence for agonist-specific changes comes from the finding that the rank order of absolute reactivity for 5-hydroxytryptamine was different to angiotensin II (Boston and Hodgson, unpublished).

In the present study, we also investigated the ability of the selective angiotensin AT_1 receptor antagonist CV-11974 to attenuate angiotensin II-mediated renal vasoconstriction. CV-11974 is reported to be a non-competitive AT_1 receptor antagonist in rabbit aorta (Noda et al., 1993). Remuzzi et al. (1993) have previously shown that chronic treatment with the angiotensin AT_1 receptor antagonist losartan prevented proteinuria and glomerulosclerosis in diabetic rats. In the present study, CV-11974 (3 μg/kg, i.v.) was chosen on the basis of preliminary experiments as a dose which would attenuate, but not abolish, angiotensin II responses so that any potential modulation of angiotensin II-induced renal vasoconstriction between treatment groups could be revealed. Indeed, at this dose CV-11974 (3 μg/kg, i.v.) caused a significant rightward shift of the angiotensin II dose-response curve in kidneys of control-SHR and control-WKY rats. However, when tested at higher doses (10 μg/kg, i.v.) in preliminary experiments, CV-11974 abolished resonses to angiotensin II confirming a non-competitive mode of action. By contrast, CV-11974 (3 μg/kg, i.v.) had no significant effect on angiotensin II responses in diabetic-SHR and -WKY rats. CV-11974 itself did not alter perfusion pressure under these conditions so alterations in baseline evoked by CV-11974 did not contribute to the present results. Kidney weights were slightly increased in diabetic groups and so it is conceivable that the diabetic rats received smaller doses of CV11974 and angiotensin II in the kidneys. However, it is unlikely that this could acount for the present findings, since kidney weights of control-WKY and diabetic-SHR rats were identical but the latter were refractory to AT_1 receptor blockade. Moreover, weights of kidneys from control-WKY rats were not significantly different from SHR rats and yet responses to angiotensin II were significantly augmented in kidneys of the latter group.

This differential modulation of angiotensin II, by CV-11974, did not appear to be dependent on either the magnitude of control angiotensin II responses (absolute or normalised) or the resting perfusion pressure in the 4 experimental groups. Indeed, it may have been anticipted that the less robust angiotensin II responses in the diabetic groups would more likely be attenuated by the AT_1 receptor antagonist, however, this in fact was not the case. Therefore, these data suggest that there may be a subtle decrease in angiotensin II receptor affinity in both diabetic groups which was only apparent from antagonist studies. Clearly, future studies are needed to address whether or not similar findings are observed using other AT_1 receptor antagonists.

In conclusion, the present study demonstrated that diabetes, uncomplicated by hypertension, is associated with a significant decrease in renal vascular responsiveness to angiotensin II, while angiotensin II responsiveness was augmented in SHR rats. However, the combination of diabetes and hypertension has largely offset the opposite effects on angiotensin II responses seen separately. Importantly, antagonist studies revealed that, at the concentration of CV-11974 used, angiotensin II responses were inhibited only in non-diabetic rats irrespective of the presence of hypertension. Overall, the results obtained may indicate alterations in angiotensin II receptor numbers (as previously reported), or angiotensin II receptor affinity in the diabetic rats, although further investigations are required.

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References

Adler, S., 1993, Diabetic nephropathy: pathogenesis and treatment, Annu. Rev. Med. 44, 303.

Ballermann, B.J., K.L. Skorecki and B.M. Brenner, 1984, Reduced glomerular angiotensin II receptor density in early untreated diabetes mellitus in the rat, Am. J. Physiol. 247, F110.

Brown, L. and C. Sernia, 1993, Angiotensin II receptors in diabetes in rats, Clin. Exp. Pharmacol. Physiol. Suppl. 1, 10.

Chukwuma, C., 1992, Comments on the clinical impact of hypertension in type 1 diabetes, J. Diabetes Complications 6, 197.

Cooper, M.E., T.J. Allen, G. Jerums and A.E. Doyle, 1986, Accelerated progression of diabetic nephropathy in the spontaneously hypertensive streptozotocin diabetic rat, Clin. Exp. Pharmacol. Physiol. 13, 655.

Cooper, M.E., T.J. Allen, R.C. O'Brien, P.A. Macmillan, B. Clarke, G. Jerums and A.E. Doyle, 1988, Effects of genetic hypertension on diabetic nephropathy in the rat – functional and structural characteristics, J. Hypertens. 6, 1009.

Cooper, M.E., T.J. Allen, R.C. O'Brien, D. Papazoglou, B. Clarke, G. Jerums and A.E. Doyle, 1990, Nephropathy in model combining genetic hypertension with experimental diabetes: enalapril versus hydralazine and metoprolol therapy, Diabetes 39, 1575.

Fioretto, P., M. Sambataro, M.G. Cipollina, E. Duner, C. Giorato, A. Morocutti, F. Mollo, G.P. Ben, A. Carraro, D. Sacerdoti, E. Brocco, G. Opocher and R. Nosadini, 1991, Impaired responses to angiotensin II in type 1 (insulin-dependent) diabetes mellitus. Role of prostaglandins and sodium-lithium countertransport activity, Diabetologia 34, 595.

Hendriks, M.C., K.L. Kam, A.J. Pijl, M. Pfaffendorf and P.A. Van Zwieten, 1993, The effects of hypertension and diabetes mellitus on the vascular reactivity of perfused mesenteric resistance arteries, J. Hypertens. 11 (Suppl. 5), S268.

Hodgson, W.C., R.G. King and A.L.A. Boura, 1990, Augmented potentiation of renal vasoconstrictor responses by thromboxane A_2 receptor stimulation in the alloxan-diabetic rat, J. Pharm. Pharmacol. 42, 423.

Kam, K.L., M. Pfaffendorf and P.A. Van Zwieten, 1994, Spontaneuosly hypertensive rats with diabetes: relaxation of isolated small arteries by various vasodilators, Can. J. Physiol. Pharmacol. 72 (Suppl. 1), 115.

Kiff, R.J., S.M. Gardiner, A.M. Compton and T. Bennett, 1991a, The effects of endothelin-1 and N^G-nitro-L-arginine methyl ester on regional haemodynamics in conscious rats with streptozotocin-induced diabetes mellitus, Br. J. Pharmacol. 103, 1321.

Kiff, R.J., S.M. Gardiner, A.M. Compton and T. Bennett, 1991b, Selective impairment of hindquarters vasodilator responses to bradykinin in conscious Wistar rats with streptozotocin-induced diabetes mellitus, Br. J. Pharmacol. 103, 1357.

Krolewski, A.S., M. Canessa, J.H. Warram, L.M.B. Laffel, A.R. Christlieb, W.C. Knowler and L.I. Rand, 1988, Predisposition to hypertension and susceptibility to renal disease in insulin-dependent diabetes mellitus, New Engl. J. Med. 318, 140.

Lentner, C., 1982, Documenta Geigy Scientific Tables, 8th edn. (Ciba Geigy, Basel) p. 210.

Lewis, E.J., L.G. Hunsicker, R.P. Bain and R.D. Rohde, 1993, The effect of angiotensin-converting enzyme inhibition on diabetic nephropathy, New Engl. J. Med. 329, 1456.

Makarious, M., K. Duggan, J.A. Charlesworth and G.J. MacDonald, 1993, Abnormality of the glomerular angiotensin II receptor in experimental diabetic nephropathy, Clin. Exp. Pharmacol. Physiol. 20, 348.

Marre, M., G. Berrut and B. Bouhanick, 1993, Hypertension and diabetes mellitus, Biomed. Pharmacother. 47, 61.

Mauer, S.M., D.M. Brown, M.W. Steffes and S. Azae, 1992, Studies of renal autoregulation in pancreatectomized and streptozotocin diabetic rats, Kid. Int. 37, 909.

Mogensen, C.E. and M.J.F. Anderson, 1975, Increased kidney size and glomerular filtration rate in untreated juvenile diabetes: normalization by insulin-treatment, Diabetologia 11, 221.

Noda, M., Y. Shibouta, Y. Inada, M. Ojima, T. Wada, T. Sanada, K. Kubo, Y. Kohara, T. Naka and K. Nishikawa, 1993, Inhibition of rabbit aortic angiotensin II (AII) receptor by CV-11974, a new nonpeptide antagonist, Biochem. Pharmacol. 46, 311.

Remuzzi, A., N. Perico, C.S. Amuchastegui, B. Malanchini, M. Mazerska, C. Battaglia, T. Bertani and G. Remuzzi, 1993, Short- and long-term effect of angiotensin II receptor blockade in rats with experimental diabetes, J. Am. Soc. Nephrol. 4, 40.

Sarubbi, D., J.C. McGiff and J. Quilley, 1989, Renal vascular responses and eicosanoid release in diabetic rats, Am. J. Physiol. 257, F762.

Soltis, E.E., 1993, Alterations in vascular structure and function after short-term losartan treatment in spontaneously hypertensive rats, J. Pharmacol. Exp. Ther. 266, 642.

Somani, P., H.P. Singh, R.K. Saini and A. Rabinovitch, 1979, Streptozotocin-induced diabetes in the spontaneously hypertensive rat, Metabolism 28, 1075.

Tomlinson, K.C., S.M. Gardiner, R.A. Hebden and T. Bennett, 1992, Functional consequences of streptozotocin-induced diabetes mellitus, with particular reference to the cardiovascular system, Pharmacol. Rev. 44, 103.

Tuncer, M. and P.M. Vanhoutte, 1991, Role of prostanoids in the increased vascular responsiveness and delayed tachyphylaxis to serotonin in the kidney of spontaneously hypertensive rats, J. Hypertens. 9, 623.

Walker, W.G., 1993, Hypertension-related renal injury: a major contributor to end-stage renal disease, Am. J. Kid. Dis. 22, 164.

Wilkes, B.M., 1987, Reduced glomerular angiotensin II receptor density in diabetes mellitus in the rat: time course and mechanism, Endocrinology 120, 1291.

Yamamoto, J., 1988, Blood pressure and metabolic effects of streptozotocin in Wistar-Kyoto and spontaneously hypertensive rats, Clin. Exp. Hypertens. 10, 1065.