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THE ROLE OF ENDOGENOUS ANGIOTENSIN II IN THE REGULATION OF RENAL HAEMODYNAMICS AND PROXIMAL FLUID REABSORPTION IN THE RAT

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SUMMARY

1. The influence of endogenous angiotensin II (AII) on renal haemodynamics and tubular function was examined by clearance and micropuncture methods in anaesthetized rats during AII receptor blockade with the non-peptide antagonist DuP 753 (50 μg kg^{-1} min^{-1} I.V.).

2. Mean arterial pressure was reduced slightly (-5 ± 2 mmHg) while filtration fraction and glomerular filtration rate rose by 30% without changes in renal plasma flow (RPF) or renal vascular resistance (RVR).

3. Fractional proximal fluid reabsorption (calculated from lithium clearance) fell from 73 to 64% ($P < 0{\cdot}01$) and fractional distal sodium reabsorption decreased from 98 to 94% ($P < 0{\cdot}01$).

4. Urine flow rate more than doubled, sodium output increased 4-fold and plasma renin concentration rose 8-fold while potassium excretion remained unchanged.

5. Proximal tubular fluid reabsorption (J_v) as measured by shrinking split-droplet micropuncture decreased by 21% ($P < 0{\cdot}01$) during infusion of DuP 753 compared with 22·5% ($P < 0{\cdot}01$) during converting enzyme inhibition by enalaprilat (MK422).

6. Responses to DuP 753 were similar to those previously documented with converting enzyme inhibitors except that DuP 753 failed to raise RPF. It is concluded that generation of intrarenal vasodilator paracrines has confounded conclusions about the renal action of converting enzyme inhibitors and we propose that in anaesthetized rats, endogenous angiotensin II (AII) has its major renal influences on glomerular filtration and proximal fluid reabsorption with little effect on renal vascular resistance.

INTRODUCTION

A role for the renin–angiotensin system in the control of renal haemodynamics and tubular transport is widely accepted but a precise definition of the intrarenal sites of action of endogenous angiotensin II (AII) and the relative importance of the various sites has been the source of continuing conjecture (Navar, Rosivall, Carmines & Oparil, 1986; Hall, 1986). Intra-arterial or intravenous infusions of exogenous AII

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decrease renal plasma flow (RPF) more than glomerula filtration rate (GFR) indicating a preferential action on postglomerular vascular resistance (Myers, Deen & Brenner, 1975; Davalos, Frega, Baker & Leaf, 1978; Edwards, 1983; Heller & Horacek, 1986). However, experiments involving suppression of endogenous AII production by angiotensin converting enzyme inhibitors (CEI), or pharmacological blockade by peptide antagonists suggest equivalent influences of AII on pre- and postglomerular vessels (Steiner & Blantz, 1979; Navar, Jirakulsomchok, Bell, Thomas & Huang, 1982; Carmines, Rosivall, Till & Navar, 1983; Harris, Zhuo & Skinner, 1987; Zhuo, Harris & Skinner, 1989).

These findings can be reconciled by asserting that exogenous AII does not accurately mimic endogenous AII in that the former raises arterial blood pressure and induces renal vasoconstriction with subsequent alteration of intrarenal vasoactive factors such as increased prostaglandin and decreased local angiotensin production which might then modulate the response of the preglomerular vessels (Baylis & Brenner, 1978; Schor, Ichikawa & Brenner, 1981; Dunn & Scharschmidt, 1987). Furthermore, deductions based on the effects of CEIs are confounded by blockade of bradykinin degradation (Ondetti & Cushman, 1982) and its attendant activation of prostaglandin synthesis (Dunn & Scharschmidt, 1987), each potentially contributing to the notable renal vasodilatation induced by CEIs (Clappison, Anderson & Johnson, 1981). The mammalian kidney possesses specific, high-affinity AII receptors located on vascular smooth muscle, glomerular mesangium, brush-border and basolateral membranes of the proximal tubule and outer medullary vascular bundles (Osbourne, Droz, Meyer & Morel, 1975; Foidart, Sraer, Delarue, Mahieu & Ardaillou, 1980; Mendelsohn, Dunbar, Allen, Chou, Millan, Aguilera & Catt, 1986; Douglas, 1987). Blockade of these endogenous angiotensin II receptors with specific antagonists should in principle clarify these issues.

Saralasin (sar^1, ala^8-AII), a competitive peptide antagonist of AII, increases RPF, GFR and glomerular ultrafiltration coefficient (K_f) without affecting filtration fraction (FF) and suppresses fluid transport in the proximal nephron (Steiner & Blantz, 1979). However, at higher doses, its partial agonist can increase blood pressure and renal vascular resistance (Case, Wallace & Laragh, 1979) and either stimulate (Spinelli & Walther, 1979) or have no effect (Schuster, Kokko & Jacobson, 1984) on proximal reabsorption. The new class of non-peptide AII receptor blockers appears to be free of agonist activity (Chiu, McCall, Price, Wong, Carini, Duncia, Wexler, Yoo, Johnson & Timmermans, 1990; Wong, Price, Chiu, Carini, Duncia, Johnson, Wexler & Timmermans, 1990*a*) and therefore promises a clearer insight into the question of the resident intrarenal influences of endogenous AII.

We have evaluated the renal haemodynamic and proximal tubular responses to a non-peptide AII receptor blocker, DuP 753, using clearance and split-drop micropuncture techniques in protocols similar to those used in our previous studies with angiotensin and CEI in anaesthetized rats (Harris *et al.* 1987; Zhuo *et al.* 1989).

METHODS

Experiments were performed on sixteen male Sprague–Dawley rats weighing 275 ± 28 g. Preparation of rats for clearance studies has been previously reported from this laboratory (Harris *et al.* 1987; Zhuo *et al.* 1989). Briefly, all rats were maintained on a normal laboratory diet (Barastoc

Stock Feeds, Melbourne, Victoria, Australia) and allowed free access to tap water until 2 days before experimentation. Each rat was then caged individually and fed a wet mash diet containing 15 mmol lithium chloride and 100 mmol sodium chloride (kg dry food)$^{-1}$. Lithium was used as a marker for proximal sodium and water reabsorption in all clearance experiments (Thomsen, 1984) and appropriate sodium intake was provided in order to minimize lithium transport beyond the end of the proximal nephron (Thomsen & Leyssac, 1986; Kirchner, 1987). The validity of the lithium clearance method as a proximal marker under such conditions has been reviewed in detail elsewhere (Thomsen, 1984; Kirchner, 1987; Koomans, Boer & Dorhout-Mees, 1989).

Rats were anaesthetized by injection of Inactin (110 mg (kg body wt)$^{-1}$ I.P.) (BYK Gulden, Konstanz, FRG) and placed on a thermostatically controlled heated table to maintain body temperature at 37 °C. A tracheostomy was performed and the right jugular vein cannulated with two catheters for infusions of 0·9% NaCl (saline), clearance markers, and drugs. Saline infusion (0·075 ml min^{-1}) began immediately after insertion of catheters. The right carotid artery was then cannulated with a polyethylene catheter (SP-35) for blood sampling and connected to a pressure transducer (model P23Db, Gould Inc., Statham Instruments, USA) and chart recorder (National, model VQ-068M, Matsushita Communication Industrial Co. Ltd, Japan). Mean arterial pressure was monitored throughout the experiment. Urine samples were collected under paraffin oil in preweighed tubes through a cannula placed in the bladder via a suprapubic midline incision.

Upon completion of surgical procedures 8% Polyfructosan (Inutest, Laevosan-Gesellschaft, Linz, Donau, Austria) and 1% *p*-aminohippuric acid (Sigma Chemical Co., St Louis, Missouri) prepared in 0·9% NaCl were initially given as a priming dose (1 ml (kg body wt)$^{-1}$ I.V.) and then infused at the rate of 2 ml h^{-1} throughout each experiment. Lithium chloride (4 mmol l^{-1} in saline) was infused from a second jugular vein catheter also at the rate of 2 ml h^{-1}. A 120 min equilibration period was allowed before the experimental protocol commenced. Rats were then divided into two groups.

Group 1: *saline time controls* ($n = 8$)

After the equilibration period, urine samples were collected every 20 min during three 60 min experimental periods and an arterial blood sample (400 μl) was taken at the midpoint of each 60 min period. Haematocrit was estimated and the plasma separated by centrifugation and stored frozen for later determination of the concentrations of electrolytes and clearance markers. The blood cells were resuspended in 200 μl saline and returned to the rat via the jugular vein catheter. The kidneys were removed, blotted, and weighed after completion of the experiment.

Group 2: *DuP 753-infused animals* ($n = 8$)

Immediately after the first 60 min period, the synthetic non-peptide AII receptor antagonist DuP 753 (2-*n*-butyl-4-chloro-5-hydroxymethyl-1-((2′-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl) imidazole, potassium salt) (E.I. du Pont de Nemours and Company, Wilmington, DE, USA) was given as a bolus dose (1 mg (kg body wt)$^{-1}$ I.V.), and then infused constantly at 50 μg (kg body wt)$^{-1}$ min^{-1} for 90 min. This dose of DuP 753 was chosen following preliminary studies in which it effectively abolished the pressor response to an intravenous injection of 5 ng angiotensin II in anaesthetized, ganglion-blocked rats (J. Zhuo, unpublished data). In the present studies, the effectiveness of receptor blockade by DuP 753 was confirmed by abolition of the pressor response to injection of 5 ng angiotensin II in 50 μl saline given immediately before and then 30 min after commencing the antagonist, and again at the end of each experiment. Urine was collected as described for Group 1 animals 30 min after equilibration periods at the initiation and termination of DuP 753 infusion. The systemic and renal effects of DuP 753 were thus observed under steady-state condition and potential dead-space errors in the urine collection precedures were minimized.

Urine output was determined gravimetrically and haematocrit measured by the microcapillary method. Sodium and potassium concentrations in plasma and urine samples were measured by flame photometry (model IL943, Instrumentation Laboratories, Lexington, MA, USA). Plasma and urine lithium concentrations were determined by atomic absorption spectrophotometry (model 901, GBC Scientific, Melbourne, Australia). Polyfructosan concentrations were estimated by the anthrone method (Fuhr, Kaczmarczyk & Kruttgen, 1955) and *p*-aminohippuric acid (pAH) as described by Smith, Finkelstein & Aliminosa (1945).

Renal clearances of polyfructosan, pAH, and lithium were calculated according to the standard

clearance equation and taken, respectively, as indices of GFR, effective renal plasma flow (ERPF), and lithium clearance (C_{Li}, representing total fluid delivery from the end of all proximal nephrons). In the absence of any evidence that DuP 753 affects renal extraction of pAH, renal plasma flow (RPF) was calculated assuming a constant 90% extraction of pAH. Fractional proximal lithium reabsorption (FR_{Li}) was calculated as the fraction of filtered lithium reabsorbed, and fractional distal reabsorption of sodium (FDR_{Na}) as the fraction of end-proximal delivery of sodium reabsorbed in the distal nephron segments. Absolute proximal fluid reabsorption (APR) was derived as $GFR - C_{Li}$.

Micropuncture studies were carried out on seventeen male Wistar–Kyoto rats (weight 280–310 g). All rats had free access to tap water and a normal laboratory diet without any lithium added. On the day of experiment, the rats were anaesthetized with Inactin (110 mg (kg body wt)$^{-1}$ I.P.) and cannulated for measurement of arterial blood pressure and for intravenous infusion of saline (4 ml h^{-1}) and drugs. The left kidney was prepared for micropuncture and proximal tubular fluid reabsorption (J_v) measured using a computerized imaging modification of the shrinking split-droplet micropuncture technique (Harris, Cullinan, Thomas & Morgan, 1987).

Upon completion of surgical preparation animals were left to equilibrate for 2 h. Three groups of animals were used (Groups 3–5) each subject to a similar protocol consisting of six 20 min periods. During the first three 20 min pretreatment periods, J_v was measured in two or three proximal convoluted tubules in each rat and a mean value obtained. At the end of the pretreatment period, rats in Group 3 ($n = 6$) received only the saline vehicle and were used as time controls, whereas those in Group 4 ($n = 6$) were treated with an angiotensin converting enzyme inhibitor, enalaprilat (5 mg (kg body wt)$^{-1}$ I.V.) (MK422, Merck, Sharp and Dohme, USA). Group 5 rats ($n = 5$) were given a single bolus dose of DuP 753 (1 mg (kg body wt)$^{-1}$ I.V.) followed by a constant infusion (50 μg (kg body wt)$^{-1}$ min^{-1}, I.V.) of the AII antagonist. In all groups, J_v was then measured again in a further two to seven tubules 30–60 min after administration of CEI or DuP 753 and a mean value calculated for each animal.

The data are presented as the mean ± S.E.M. Student's paired t test was used to evaluate differences between the pretreatment and experimental periods within each group of rats in both lithium and micropuncture studies. Student's unpaired t test was used to assess the differences between the corresponding periods of Groups 1 and 2, while in micropuncture studies, comparisons between groups were made using one-way ANOVA followed by single degree of freedom planned comparisons. Statistical significance was determined at $P < 0.05$.

RESULTS

Systemic effects of DuP 753

In rats receiving DuP 753 (Group 2), mean arterial pressure (MAP) decreased slightly from 129 ± 2 to 124 ± 4 mmHg ($P < 0.01$) but remained low (120 ± 3 mmHg, $P < 0.01$) after termination of DuP 753 infusion indicating the long-acting nature of this AII antagonist. MAP remained stable throughout the pretreatment and experimental periods in the saline time controls (Group 1). DuP 753 did not alter haematocrit or plasma concentrations of sodium, potassium or clearance markers in either Group 1 or Group 2 animals.

Renal effects of DuP 753

Blockade of angiotensin receptors by DuP 753 (Group 2) resulted in marked glomerular hyperfiltration. As illustrated in Fig. 1, GFR increased by 29% and, compared with control, remained elevated 30 min after infusion of DuP 753 ended. However, DuP 753 had no effect on renal plasma flow or calculated renal vascular resistance (RVR). Since GFR increased without an accompanying rise in RPF, filtration fraction was consequently elevated. In the time controls (Group 1), renal haemodynamic parameters were unaltered.

As shown in Fig. 2, infusion of DuP 753 (Group 2) caused marked diuresis and

natriuresis without affecting potassium excretion. Urine flow rate (V) more than doubled whereas urinary sodium excretion ($U_{Na}V$) increased by 278%. Fractional water excretion (FE_{H_2O}) increased by 100% and fractional sodium excretion (FE_{Na}) by 250%. Diuresis and natriuresis were still evident 30 min after cessation of DuP

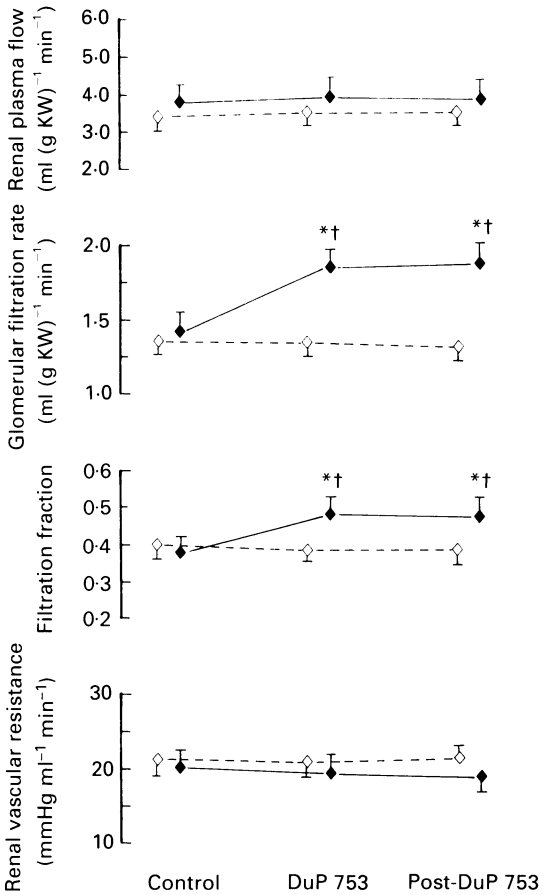

Fig. 1. Renal haemodynamic responses to saline vehicle (Group 1, ◇) and non-peptide angiotensin II receptor antagonist, DuP 753 (Group 2, ◆). Values are averages from three 20 min urine collection periods. Control, pretreatment period; DuP 753, period of constant infusion of the antagonist at 50 μg (kg body wt)$^{-1}$ min^{-1} following a bolus dose of 1 mg (kg body wt)$^{-1}$ I.V.; Post-DuP 753, period starting 30 min after infusion of DuP 753 was terminated. *, significant difference ($P < 0{\cdot}01$) between control and experimental periods within a group; †, significant difference ($P < 0{\cdot}01$) between corresponding periods of Groups 1 and 2. KW, kidney weight.

753 infusion. Renal electrolyte and water excretion showed no significant variations in the time-control animals (Group 1).

The effects of DuP 753 on proximal and distal tubular reabsorption estimated by lithium clearance are presented in Fig. 3. DuP 753 markedly increased solute and fluid delivery (estimated as C_{Li}) from the proximal tubule into the distal segments of the nephron by 68%, indicating inhibition of proximal tubule sodium reabsorption.

Provided that DuP 753 does not promote significant distal lithium reabsorption, the observed increase in C_{Li} after AII receptor blockade is consistent with a 9% fall in fractional proximal fluid reabsorption (estimated from FR_{Li}). DuP 753 also suppressed the estimated fractional distal reabsorption of sodium. While GFR

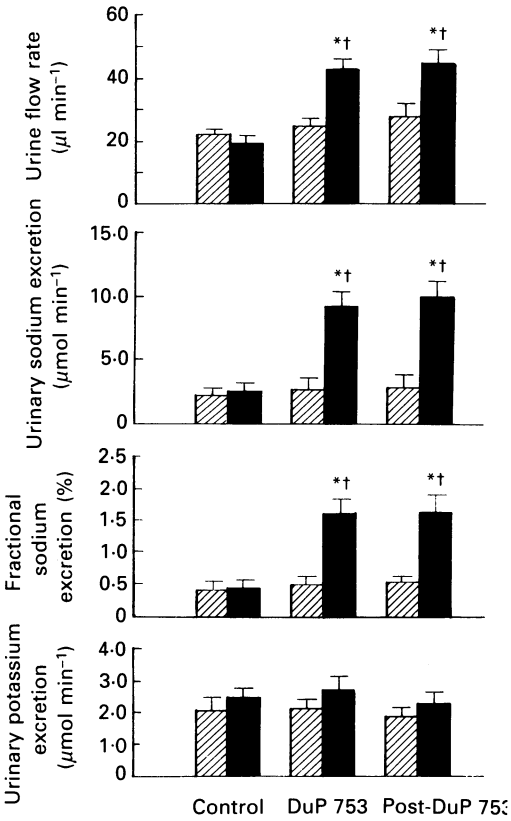

Fig. 2. Bar graphs showing changes in urine flow rate, urinary and fractional sodium excretion, and urinary potassium excretion in saline time controls (Group 1, ▨) and in rats receiving infusion of DuP 753 (Group 2, ■). Abbreviations and levels of significance as in Fig. 1.

increased by 29% APR rose by only 15% revealing an impairment of proximal glomerulo-tubular balance during infusion of the AII antagonist.

In contrast with the prolonged haemodynamic actions of DuP 753, the reduction in fractional proximal reabsorption was reversed within 60 min after termination of the antagonist infusion. APR increased and the effectiveness of proximal GTB improved from 51% during DuP 753 infusion to 71%. However, compared with control, FDR_{Na} remained suppressed during the recovery period. In the time-control animals (Group 1) renal tubular function showed no significant variations.

As shown in Fig. 4, blockade of AII receptors with DuP 753 was accompanied by an average 8-fold increase in plasma renin concentration in Group 2. By comparison, plasma renin concentration rose by less than 3-fold in Group 1 time controls.

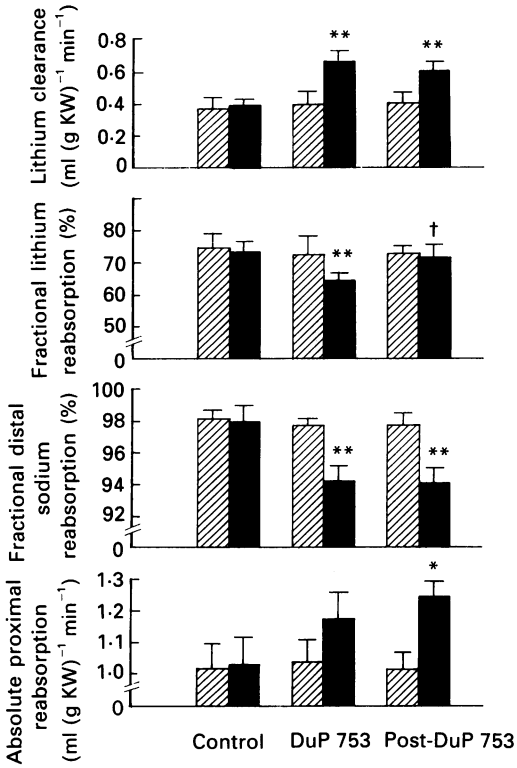

Fig. 3. Lithium clearance and renal tubular transport responses to saline vehicle (Group 1, ▨) and infusion of DuP 753 (Group 2, ■). Differences from the control period within a group and between the corresponding periods of Groups 1 and 2 are shown as * ($P < 0·05$) or ** ($P < 0·01$) and between consecutive experimental periods within Group 2 as † ($P < 0·05$).

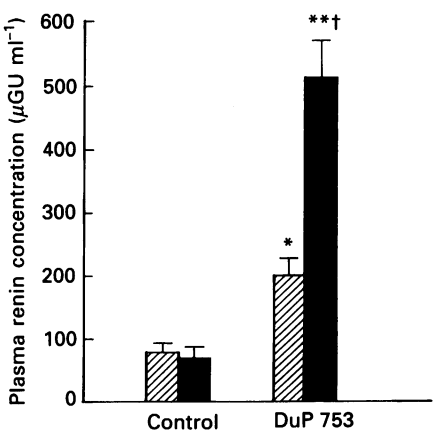

Fig. 4. Bar graphs showing changes in plasma renin concentration during infusion of saline vehicle (Group 1, ▨) and DuP 753 (Group 2, ■). Differences between control and experimental periods within a group are shown as * ($P < 0·05$) or ** ($P < 0·01$) and between corresponding periods of Groups 1 and 2 as † ($P < 0·01$). GU, Goldblatt units.

Micropuncture studies

In all three groups studied by micropuncture (Groups 3–5), no significant differences were observed in MAP or J_v during the first three control periods (Table 1). Subsequent intravenous infusions of enalaprilat (Group 4) or DuP 753 (Group 5) caused similar decreases in MAP. Mean J_v was reduced by 20·6% and 22·5% in

TABLE 1. Effects of enalaprilat and non-peptide AII receptor antagonist DuP 753 on mean arterial pressure (MAP) and proximal fluid reabsorption (J_v) in anaesthetized rats

	Group 3 ($n = 6$) Time control		Group 4 ($n = 6$) Enalaprilat		Group 5 ($n = 5$) DuP 753	
	C	E	C	E	C	E
MAP (mmHg)	97 ± 3	98 ± 3	103 ± 4	83 ± 4*	108 ± 6	92 ± 5*
J_v ($\times 10^{-4}$ mm^3 mm^{-2} s^{-1})	3·64 ± 0·17	3·72 ± 0·12	3·74 ± 0·1	2·99 ± 0·17*	3·76 ± 0·16	2·90 ± 0·15*

C, control period; E, experimental period. * $P < 0·01$ significantly different from control.

animals treated with enalaprilat and DuP 753 respectively, but remain unchanged in time controls (Group 3).

DISCUSSION

DuP 753 has been shown to inhibit specific binding of labelled AII to receptor sites and to competitively antagonize AII-induced contraction of vascular smooth muscle and aldosterone release without discernible agonist activity (Chui *et al.* 1990; Wong *et al.* 1990*a*). DuP 753 infused at 50 μg kg^{-1} min^{-1} effectively abolished the systemic pressor response to injection of 5 ng AII throughout the present experiments demonstrating at least systemic and probably therefore intrarenal blockade of endogenous AII activity. DuP 753 inhibited proximal tubular fluid reabsorption and increased GFR and urinary excretion of sodium and water but it did not alter RPF or calculated RVR. These responses indicate that in anaesthetized rats endogenous AII experts a powerful influence on glomerular filtration with apparently only minor effects on intrarenal vascular resistance. In addition, we found that the hyperfiltration and systemic hypotensive effects of DuP 753 persisted for at least 60 min after cessation of the infusion but that the proximal tubular response reversed within 30 min. Persistence of haemodynamic effects is consistent with the studies of Wong, Price, Chiu, Duncia, Carini, Wexler, Johnson & Timmermans (1990*b*) who reported a long-acting antihypertensive action of this antagonist.

Comparisons with previous studies from this laboratory (Harris *et al.* 1987; Zhuo *et al.* 1989) show that the effects of DuP 753 on glomerular filtration and renal electrolyte excretion are similar to those produced by blockade of angiotensin converting enzyme with enalaprilat. However, the lack of any detectable change in renal plasma flow as indicated by pAH clearance is a major difference between our findings with these two agents.

Navar *et al.* (1982) reported that CEI causes decreases of approximately 30% in preglomerular and postglomerular resistances in anaesthetized dogs and concluded that endogenous AII exerts similar degrees of vasoconstriction in afferent and

efferent arterioles. Conversely, infusion of exogenous AII elicits dose-dependent decreases in RBF consistent with renal vasoconstriction (Myers *et al.* 1975; Hall, 1986). However, the concomitant effects on GFR are proportionally smaller such that FF increases indicating that exogenous AII has a primary action on postglomerular vascular resistance. The extent to which these apparently conflicting observations might result from preservation of vasodilator kinins in renal tissue following kininase II blockade by CEI (Clappison *et al.* 1981), or to increased prostaglandin production during AII infusion (Dunn *et al.* 1987), may be resolved by comparison with responses to a more specific AII receptor blocker such as DuP 753.

In the present study, the renal haemodynamic responses to infusion of DuP 753 differ importantly from those cited above with CEI or saralasin or by extrapolation from the effects of infused AII. DuP 753 increased GFR by 29% whereas RPF and calculated RVR remained unaltered and FF was consequently elevated by 30% (Fig. 1). A lack of effect of DuP 753 on renal vasculature has also been seen in the perfused isolated rat kidney where it blocked the vasoconstrictor action of AII but did not affect RVR (Fontoura, Nussenzveig, Timmermans & Maack, 1990). It is possible to explain the glomerular response of DuP 753 in terms of postglomerular arteriolar constriction accompanied by preglomerular dilatation, thus keeping RVR constant while raising FF. There is, however, no experimental support for such a scheme. DuP did not alter MAP to a level that would activate renal autoregulatory responses to any important extent, and unlike the peptide antagonist saralasin, it does not have AII-like vasoconstrictor effects (Wong *et al.* 1990*a*). DuP 753 appears to remove selectively the influence of endogenous AII which in turn leads to an increase in GFR. Since AII has powerful contractile effects in the glomerular mesangium (Osbourne *et al.* 1975; Foidart *et al.* 1980) that reduce the ultrafiltration coefficient (K_f), specific blockade of mesangial AII receptors by DuP 753 may increase K_f, leading to hyperfiltration without raising filtration pressure. Other renal vasoactive systems may underly the conflicting renal haemodynamic actions of AII found in studies using infusions of exogenous AII or suppression of endogenous AII by CEI (Dunn & Scharschmidt, 1987; Tucker & Blantz, 1990).

DuP 753 also induced diuresis and natriuresis to an extent similar to that caused by CEI in previous experiments (Harris *et al.* 1987; Zhuo *et al.* 1989; Xie, Liu, Wong, Timmermans & Cogan, 1990) although in the study by Xie *et al.* (1990) whole kidney GFR did not rise with either DuP 753 or catopril despite increased values for single nephron GFR (SNGFR). In contrast to the data presented here, no acute diuretic effect of this agent was observed in spontaneously hypertensive rats (Wong *et al.* 1990*b*), probably due to the more pronounced fall in MAP in hypertensive compared with normotensive rats.

A proximal tubular action of DuP 753 was indicated in the present study by the lithium clearance and micropuncture data. DuP 753 markedly increased C_{Li} (68%), indicating increased end-proximal fluid delivery into the distal nephron segments. Calculated fractional lithium reabsorption (FR_{Li}) fell by 9%, consistent with reduced fractional proximal sodium and water reabsorption. Although absolute proximal reabsorption (APR) increased in response to the enhanced filtered load, the rise in APR (15%) was less than for GFR (29%). Proximal glomerulo-tubular balance (PGTB) was therefore only 51% effective compared with 'perfect' balance

characterized by parallel changes in GFR and APR (Zhuo *et al.* 1989). The extent of inhibition by DuP 753 of proximal sodium and water transport and of PGTB as derived from lithium and inulin clearance measurements is similar to that observed previously with CEI (Harris *et al.* 1987; Zhuo *et al.* 1989).

In addition to the clearance data that were strongly indicative of suppression of a proximal action of AII the micropuncture measurements reported here provide more direct evidence that removal of endogenous AII results in a reduction (23%) in proximal tubular fluid reabsorption (J_v). Similar responses were observed in our previous micropuncture studies in which CEI reduced proximal reabsorption by 25–30% (Harris, Navar & Ploth, 1984; Thomas, Harris & Morgan, 1988) most likely reflecting withdrawal of the stimulatory action of AII on proximal sodium transport (Harris & Young, 1977; Schuster *et al.* 1984). Xie *et al.* (1990) have recently reported that DuP 753 reduces bicarbonate, chloride and water reabsorption in S1 and S2 segments of the Munich–Wistar rat and note that in the S1 subsegment DuP 753 was more effective than captopril.

An intriguing facet of the present study is that the effect of DuP 753 on proximal fluid transport was less persistent than on distal nephron segments or on glomerular filtration. The reduction in fractional proximal reabsorption (FR_{Li}) was reversed within 30 min after termination of DuP 753 infusion and PGTB improved, albeit to only 71% effectiveness. At this time, GFR and FF remained elevated, fractional distal sodium reabsorption remained depressed and diuresis and natriuresis continued. The basis for the differential time courses of these effects in the tubule and glomerulus is unknown but may involve differences in either the receptors or signal transduction mechanisms. In pharmacological studies, selective AT-1 antagonists including DuP 753 have been used to distinguish AII receptor subtypes and to date, only AT-1 receptors have been identified in kidney (de Gasparo, Whitebread, Mele, Montani, Whitcombe, Ramjoue & Kamber, 1990). Douglas (1987) has emphasized that there are at least two distinct functional classes (Types A and B) of angiotensin receptors in the kidney cortex perhaps representing a further subdivision of the AT-1 subtype. Type A receptors are localized to the glomerular mesangium and signal transduction is mediated by phospholipase C (Pfeilschifter & Bauer, 1986; Douglas, 1987). Type B receptors occur at a lower density in the outer cortex corresponding to proximal tubular epithelium (de Gasparo *et al.* 1990) and signal transduction appears to be mediated by adenylate cyclase activity such that a reduced intracellular cAMP levels is associated with increased luminal Na^+–H^+ exchange and stimulation of sodium reabsorption (Douglas, 1987; Liu & Cogan, 1987). The proximal tubule effects of DuP 753 might therefore involve removal of a resident action of AII from Type B receptors resulting in decreased sodium uptake.

DuP 753 administration was accompanied by an average 8-fold increase in plasma renin concentration, similar to that reported previously with CEI (Harris *et al.* 1987) and presumably the result of removal of the 'short-loop negative feedback' of AII on juxtaglomerular cells (Kurtz & Penner, 1989). Neither absolute nor fractional excretion of potassium was significantly increased by DuP 753 despite enhanced delivery of filtrate to the distal nephron, consistent with the findings of Xie *et al.* (1990). A possible explanation is that aldosterone secretion decreased as a consequence of the fall in plasma renin concentration.

We conclude that DuP 753 and similar angiotensin blockers can provide new insight into the endogenous actions of AII and III and promise to be useful tools in the study of AII receptor subtypes in the kidney. The data presented do not support the proposal that intrarenal angiotensin is active in maintaining glomerular vascular resistance but indicate that, at least in anaesthetized rats, its primary effects are mediated via glomerular capillary permeability or filtration surface area and proximal and perhaps also distal tubule sodium and water reabsorption.

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