Cardiac haemodynamic effects of the non-peptide, angiotensin II-receptor antagonist, DuP 753, in conscious Long Evans and Brattleboro rats

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1 In previous experiments in conscious, water-replete Long Evans and Brattleboro rats the non-peptide angiotensin II-receptor antagonist, DuP 753, caused only slight hypotension and peripheral (particularly renal) vasodilatations. However in water-deprived (i.e. renin-dependent) Brattleboro rats, DuP 753 caused marked hypotension and regional vasodilatations. The major objective of the present study was to determine if the hypotensive effects of DuP 753 under any of the experimental conditions studied previously were contributed to by negative effects on cardiac haemodynamics.

2 Male, Long Evans and Brattleboro rats were chronically instrumented with electromagnetic flow probes on the ascending aorta and with intravascular catheters. Data were collected by use of a microcomputer-based system that provided digitised print-out of instantaneous heart rate, mean arterial blood pressure, cardiac output, stroke volume, peak aortic flow, maximum positive slope of the aortic flow signal ($+dF/dt_{\max}$), total peripheral conductance and central venous pressure.

3 Incremental i.v. bolus doses (0.1 – 10 mg kg^{-1} , at 15 min intervals) of DuP 753 were administered to water-replete Long Evans ($n = 8$) and Brattleboro ($n = 8$) rats, and to water-deprived (14 h) Brattleboro rats ($n = 9$) (the latter animals show marked activation of the renin-angiotensin system). In all groups, 15 min after the highest dose of DuP 753 had been given, a supramaximal dose of captopril (2 mg kg^{-1}) was injected to determine if it had any additional effects.

4 In water-replete, Long Evans and Brattleboro rats, DuP 753 (0.1 – 1 mg kg^{-1}) caused slight, transient hypotension, with rises in total peripheral conductance; increases in cardiac output, peak aortic flow, $+dF/dt_{\max}$ and stroke volume were inconsistent, and central venous pressure did not change. Higher doses of DuP 753 (3 and 10 mg kg^{-1}) caused modest, sustained hypotension that was due entirely to an increase in total peripheral conductance, since cardiac output, peak aortic flow and $+dF/dt_{\max}$ showed transient elevations; captopril had no additional hypotensive or vasodilator effects.

5 Under resting conditions, water-deprived Brattleboro rats showed an increase in mean arterial blood pressure, but there were reductions in total peripheral conductance, cardiac output and $+dF/dt_{\max}$. DuP 753 (0.1 – 10 mg kg^{-1} , i.v., boluses at 15 min intervals) caused incremental hypotension and tachycardia and dose-dependent rises in total peripheral conductance that were accompanied by transient increases in cardiac output, peak aortic flow and $+dF/dt_{\max}$; captopril had no additional hypotensive or vasodilator effects.

6 Under no conditions were there any negative effects of DuP 753 on myocardial function; moreover, it is likely the transient increases in cardiac function following DuP 753 were indirect and due to the reduction in afterload. Thus, it appears the haemodynamic effects of DuP 753 can be explained by its vasodilator action.

Keywords: Cardiac haemodynamics; non-peptide angiotensin II-receptor antagonist; DuP 753

Introduction

Our understanding of the involvement of the renin-angiotensin system (RAS) in cardiovascular regulation has been advanced greatly by the availability of angiotensin converting enzyme (ACE) inhibitors such as captopril, and antagonistic peptide analogues of angiotensin II (AII) such as saralasin. However, results obtained with these pharmacological tools *in vivo* may not be clear-cut since ACE inhibitors can affect the metabolism of several peptides in addition to inhibiting the generation of AII (Zusman, 1984; Skidgel *et al.*, 1987) and the antagonistic peptide analogues of the latter also have agonistic effects (see Tomlinson *et al.*, 1990).

Recently it has been demonstrated that there are at least two sub-types of AII receptors. The AII-receptor sub-type associated with vascular smooth muscle (originally described as AII-B (Whitebread *et al.*, 1989) or AII₁ (Chiu *et al.*, 1989)) is selectively blocked by compounds such as 2-n-butyl-4-chloro-5-hydroxymethyl-1-[2-(1H-tetrazole-5-yl)bi-phenyl-4-yl

methyl]imidazole, potassium salt (DuP 753) (Chiu *et al.*, 1990; Wong *et al.*, 1990b,c,d,e,f). DuP 753 is totally lacking in agonistic effects and has no influence on vascular responses to nor-adrenaline, vasopressin or bradykinin, or on ACE activity (Chiu *et al.*, 1990; Wong *et al.*, 1990b,c,d,e,f).

In conscious, normotensive, Sprague-Dawley rats, or in rats with DOCA-salt hypertension, DuP 753 has no hypotensive effect, but in normotensive Sprague-Dawley rats treated with frusemide, in rats with hypertension due to renal artery ligation and in spontaneously hypertensive rats, DuP 753 lowers blood pressure (Wong *et al.*, 1990b,c,d,e,f). In those studies detailed haemodynamic measurements were not made, leaving open the possibility that DuP 753 might influence regional vascular conductances and/or cardiac output, even in conditions in which mean arterial blood pressure was unaffected. In order to cover the former possibility we examined the regional haemodynamic effects of DuP 753 in conscious, water-replete, Long Evans and Brattleboro rats (Batin *et al.*, 1991). We chose these animals because we have substantial background information on them and, more importantly, cardiovascular status in Brattleboro rats can be made dependent on the RAS

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simply by depriving them of drinking water for a short time (Gardiner & Bennett, 1985; Bennett & Gardiner, 1987; Tomlinson *et al.*, 1990; Muller *et al.*, 1990).

In water-replete, Long Evans and Brattleboro rats, DuP 753 had little effect on mean arterial blood pressure, although it did cause mesenteric and renal vasodilatations. The absence of a change in mean arterial blood pressure when renal and mesenteric vasodilatations occurred, indicates that cardiac output might have risen along with vascular conductances, but no direct measurement of cardiac haemodynamics was possible in those animals since they were extensively instrumented for recording renal, mesenteric and hindquarters blood flows. Therefore, the first aim of the present study was to make direct measurements of cardiac haemodynamics in water-replete, Long Evans and Brattleboro rats and to assess changes in those variables in response to incremental i.v. bolus doses of DuP 753 to match our previous protocol (Batin *et al.*, 1991). This protocol was chosen originally because DuP 753 has an extremely long duration of action (Wong *et al.*, 1990c,d,e; Batin *et al.*, 1991) and hence it is not possible to avoid cumulative effects with any reasonable dosing cycle.

In water-deprived Brattleboro rats with marked activation of the RAS, captopril causes substantial hypotension (Gardiner & Bennett, 1985; Tomlinson *et al.*, 1990; Muller *et al.*, 1990). Consistent with these findings, administration of DuP 753 to water-deprived Brattleboro rats caused renal, mesenteric and hindquarters vasodilatations together with a sizeable fall in mean arterial blood pressure (Batin *et al.*, 1991). However, in those experiments we could not exclude the possibility that inhibition of the cardiotoxic effects of AII (Urata *et al.*, 1990; Hirakata *et al.*, 1990; Lindpaintner *et al.*, 1990) contributed to the hypotensive action of DuP 753. Therefore, the second aim of the present study was to measure changes in cardiac haemodynamics in water-deprived Brattleboro rats given DuP 753, following the same protocol as previously (Batin *et al.*, 1991).

As mentioned above, it is feasible that the acute cardiovascular effects of captopril involve mechanisms in addition to inhibition of AII production. If this were the case then administration of captopril in the presence of a maximally effective dose of DuP 753 should cause additional cardiac haemodynamic changes. Our third aim was to investigate this possibility, as we had in our previous investigation involving measurement of regional haemodynamics (Batin *et al.*, 1991).

Methods

Male, Long Evans and homozygous Brattleboro rats (350–450 g) were anaesthetized (sodium methohexitone, 60 mg kg⁻¹ i.p., supplemented as required) and had an electromagnetic flow probe (Skalar, Delft, Netherlands) implanted around the ascending thoracic aorta (Smith & Hutchins, 1979; Smits *et al.*, 1982; Gardiner *et al.*, 1990a,b,c). Animals were given ampicillin (7 mg kg⁻¹ i.m.; Penbritin, Beechams) after surgery and kept in their home cages for at least 7 days. Thereafter, under brief anaesthesia (sodium methohexitone 40 mg kg⁻¹, i.p.) intravascular catheters were implanted; the tip of an intraarterial catheter was positioned in the distal abdominal aorta (via the ventral caudal artery), and 3 catheters were implanted in the right jugular vein. One of the venous catheters was placed with its tip near the right atrial orifice to allow the measurement of central venous pressure; this catheter was infused continuously with saline (NaCl 154 mmol l⁻¹) to prevent emboli forming at its tip (Gardiner *et al.*, 1990a,b,c). All catheters were tunnelled subcutaneously to emerge at the back of the neck close to the flow probe connector. The catheters were contained within a flexible spring connected to a harness worn by the rat. A short interconnecting cable from the flow probe was taped to the spring to allow connections to be made to the flowmeter (Skalar, MDL 1401) without disturbing the animal. The whole assembly was supported by a counter-balanced lever system (Gardiner *et al.*, 1990a,b,c). Animals

were returned to their home cages and left to recover for at least 48 h before experiments were begun. During this time Long Evans rats were infused with an additional 20 ml of isotonic saline each day (at 1 ml h⁻¹) in order to avoid any possible problems with post-surgical hypodipsia. The latter phenomenon is not observed in Brattleboro rats since their usual polydipsia is re-established very soon after surgery (unpublished observations).

During an experiment, the inputs from the thoracic aortic flow probe and intraarterial and central venous pressure transducers were fed into a custom-built haemodynamics microprocessor (University of Limburg Instrument Services and Pharmacology Departments) interfaced with a Tandon 386 microcomputer. This system digitized the input signals every 2 ms and stored data every 1 or 10 s to disc, as required. Off-line, selected portions of the experiment were analysed and average values for mean arterial blood pressure, heart rate, cardiac output, stroke volume, peak aortic flow, maximum rate of positive change of aortic flow ($+dF/dt_{\max}$) and central venous pressure were obtained. (Peak aortic flow and $+dF/dt_{\max}$ have been taken as indirect indices of contractility (Schoemaker, 1989)). The cardiac variables were not normalised for body weight since water deprivation in Brattleboro rats causes substantial loss of body weight (Gardiner & Bennett, 1985). However, in the water-replete state there were no differences in body weight in the different groups of animals.

All experiments began between 06 h 30 min and 07 h 00 min. The water-deprived Brattleboro rats had had their drinking water removed at 17 h 00 min the previous day and hence had been without water for 14 h. This protocol produces about 25% reduction in plasma volume and makes systemic arterial blood pressure very sensitive to the hypotensive effects of captopril (Gardiner & Bennett, 1985; Tomlinson *et al.*, 1990; Muller *et al.*, 1990).

As mentioned in the Introduction, DuP 753 has a very prolonged action (Wong *et al.*, 1990c,d,e; Batin *et al.*, 1991), and hence it is not possible to avoid cumulative effects. Therefore, in our previous study (Batin *et al.*, 1991) we chose to give incremental i.v. bolus doses of DuP 753 (0.1–10 mg kg⁻¹) at 15 min intervals, as did Wong *et al.* (1990c,d,e). In the present experiments we used the same protocol as previously since we wanted to obtain cardiac haemodynamic data to match the regional haemodynamic data obtained earlier (Batin *et al.*, 1991).

A dose of 10 mg kg⁻¹ DuP 753 has a maximal effect (Wong *et al.*, 1990d,e; Batin *et al.*, 1991), that causes complete inhibition of the regional haemodynamic responses of exogenous AII (50 ng) for at least 9 h (Batin *et al.*, 1991). Therefore 15 min after the 10 mg kg⁻¹ dose of DuP 753 a supramaximal dose of captopril (2 mg kg⁻¹; Muller *et al.*, 1990) was given to assess if it had any additional cardiac haemodynamic effects in the presence of DuP 753, as we had in our previous study when regional haemodynamics only were measured.

All injections were given in 0.1 ml and flushed in with 0.1 ml saline (dead space of the catheter). DuP 753 and captopril were dissolved in isotonic saline; injection of isotonic saline alone had no significant cardiovascular effects, and over the time course of the experiment (i.e. 90 min) there were no significant changes in any cardiovascular variable if DuP 753 was not administered. We did not carry out 'placebo' experiments since the purpose of the investigation was to determine if any negative changes in cardiac function contributed to the hypotensive action of DuP 753 under any condition.

Experiments were carried out in water-replete, Long Evans ($n = 8$) and Brattleboro ($n = 8$) rats and water-deprived ($n = 9$) Brattleboro rats.

Data analysis

Data were analysed by Friedman's test (Theodorsson Norheim, 1987) for within-group comparisons. In order to assess the acute effects of DuP 753, the changes over the first

5 min following each dose were compared to the immediate pre-injection value. In addition, over the time course of the experiment each pre-injection value was compared to the original pre-drug baseline. Between group comparisons were made with the Kruskal-Wallis test or Mann-Whitney U test, as appropriate.

Drugs

DuP 753 (gift from Dr R. Smith, DuPont, U.S.A.) and captopril (gift from Squibb, U.S.A.) were dissolved in isotonic saline.

Results

Resting cardiovascular variables

In the resting state there were no significant differences between cardiovascular variables in water-replete Long Evans and Brattleboro rats. However, following water deprivation, Brattleboro rats showed an increase in mean arterial blood pressure accompanied by decreases in cardiac output, $+dF/dt_{\max}$, and total peripheral conductance (Table 1).

Responses to DuP 753 in water-replete Long Evans rats

The lower doses of DuP 753 ($0.1\text{--}1\text{ mg kg}^{-1}$) caused slight, transient falls in mean arterial pressure accompanied by rises in total peripheral conductance, but no consistent changes in the other cardiovascular variables (Figure 1). Doses of 3 and 10 mg kg^{-1} DuP 753 caused small, but sustained, falls in mean arterial blood pressure, and increases in heart rate and total peripheral conductance (Figure 1). There were transient increases in cardiac output, peak aortic flow and $+dF/dt_{\max}$, but not in stroke volume or central venous pressure (Figure 1). Administration of captopril (2 mg kg^{-1}) 15 min after the highest dose of DuP 753 caused a slight tachycardia and a transient rise in mean arterial blood pressure, accompanied by brief increases in peak aortic flow and $+dF/dt_{\max}$ (Figure 1).

Responses to DuP 753 in water-replete Brattleboro rats

DuP 753 ($0.1\text{--}10\text{ mg kg}^{-1}$) caused effects similar to those seen in Long Evans rats. Thus, there were incremental reductions (albeit slight) in mean arterial blood pressure associated with rises in total peripheral conductances and heart rate (Figure 2). There were no sustained changes in cardiac output, stroke

volume, peak aortic flow, $+dF/dt_{\max}$ or central venous pressure (Figure 2). Furthermore, after the 10 mg kg^{-1} dose of DuP 753, captopril had no additional effects (Figure 2).

Responses to DuP 753 in water-deprived Brattleboro rats

The lower doses of DuP 753 ($0.1\text{--}1\text{ mg kg}^{-1}$) caused transient falls in mean arterial blood pressure accompanied by brief increases in heart rate, cardiac output, stroke volume, peak aortic flow and $+dF/dt_{\max}$ (Figure 3). The higher doses of DuP 753 (3 and 10 mg kg^{-1}) caused sustained hypotension and tachycardia, together with transient increases in cardiac output, peak aortic flow, $+dF/dt_{\max}$ and total peripheral conductance (Figure 3). However, stroke volume fell relative to the pre-drug baseline and there were no consistent changes in central venous pressure (Figure 3). Administration of captopril (2 mg kg^{-1}) 15 min after DuP 753 (10 mg kg^{-1}) caused a slight tachycardia, but no other significant effects (Figure 3).

Relative to the pre-drug baseline values, 15 min after administration of DuP 753 at a dose of 10 mg kg^{-1} , the falls in mean arterial blood pressure and the rises in total peripheral conductance and peak aortic flow were significantly greater in water-deprived than in water-replete Brattleboro rats (Table 2).

Discussion

In the present work we investigated the cardiac haemodynamic effects of DuP 753 in conscious rats primarily to determine if, under any experimental condition in which we had previously studied DuP 753 (Batin *et al.*, 1991), there were signs of negative changes in myocardial function that could have contributed to the effects of DuP 753 on mean systemic arterial blood pressure. For this reason we employed the protocol used previously, both by us (Batin *et al.*, 1991) and Wong *et al.* (1990c,d,e), involving incremental i.v. bolus doses of DuP 753 at 15 min intervals. Although this protocol does not produce sustained cardiovascular effects with the lower doses of DuP 753, it is likely the recovery is due to increased activity of the RAS, rather than a waning of drug action, since the effects following administration of DuP 753 are rapid in onset and prolonged (Wong *et al.*, 1990c,d,e; Batin *et al.*, 1991). Recently Wong *et al.* (1990e) reported that, after administration of DuP 753 *in vivo*, an active metabolite (EXP 3174) was formed. EXP 3174 is more potent than DuP 753 and is a non-competitive antagonist at the AII_1 receptor (Wong *et al.*,

Table 1 Resting cardiovascular variables in conscious rats

	Water-replete		Water-deprived
	Long Evans (n = 8)	Brattleboro (n = 8)	Brattleboro (n = 9)
Heart rate (beats min^{-1})	381 ± 8	358 ± 11	$330 \pm 10^\dagger$
Mean arterial blood pressure (mmHg)	100 ± 2	100 ± 4	$113 \pm 1^\dagger^*$
Cardiac output (ml min^{-1})	107 ± 6	109 ± 6	$88 \pm 3^\dagger^*$
Peak aortic flow (ml min^{-1})	411 ± 22	432 ± 26	387 ± 16
$+dF/dt_{\max}$ (l min^{-2})	1773 ± 104	1784 ± 140	$1605 \pm 89^\dagger^*$
Stroke volume ($\mu\text{l beat}^{-1}$)	283 ± 17	305 ± 19	269 ± 12
Total peripheral conductance ($\text{ml min}^{-1} \text{ mmHg}^{-1}$)	1083 ± 69	1102 ± 85	$780 \pm 28^\dagger^*$
Central venous pressure (cmH_2O)	3.3 ± 0.9	3.8 ± 0.6	4.9 ± 0.4

Values are mean $\pm$ s.e.mean.

$^\dagger P < 0.05$ versus water-replete Long Evans rats.

$^* P < 0.05$ versus water-replete Brattleboro rats.

Figure 1 Cardiovascular variables in conscious, water-replete, Long Evans rats ($n = 8$). At the arrows between 0 and 60 min, increasing i.v. bolus doses (0.1–10 mg kg⁻¹) of DuP 753 were administered (i.e. at 15 min intervals). Fifteen min after the highest dose of DuP 753 a supramaximal dose of captopril (2 mg kg⁻¹) was administered. The values shown are the means, and the bars represent s.e. means; where the bars are not visible they lie within the symbol. * $P < 0.05$ versus pre-injection value for that dose; † $P < 0.05$ versus original pre-drug baseline. For clarity this latter comparison was made only for the 15 min post-injection values. The dashed line corresponds to the original baseline.

1990e), and hence could contribute to the effects following administration of DuP 753. While it could be argued that, in the present study, the changes in cardiac haemodynamics with increasing doses of DuP 753 were due to cumulative generation of EXP 3174, it is notable that the profiles of effect of DuP 753 and EXP 3174 on mean arterial blood pressure are similar (Wong *et al.*, 1990c,d,e,f). This observation lends support to the suggestion that the apparent recovery from the effects of the lower doses of DuP 753 was due to additional activation of the RAS. Although there is a theoretical possibility that AII could act on AII₂ receptors to influence cardiovascular function in the presence of DuP 753, Wong *et al.* (1990b) were unable to identify a functional role for such receptors. Moreover, in water-deprived Brattleboro rats, administration of PD 123177 (a selective antagonist for AII₂ receptors (Wong *et al.*, 1990b)) at doses up to 100 mg kg⁻¹

had no haemodynamic effects (Gardiner, S.M. *et al.*, unpublished observations).

Cardiac haemodynamic changes following DuP 753 in water-replete rats

Consistent with our previous findings of renal and mesenteric vasodilator responses to the lower doses of DuP 753 in water-replete, Long Evans and Brattleboro rats, the present study showed transient increases in total peripheral conductance following the same intervention. As we suspected, (Batin *et al.*, 1991) there were transient rises in cardiac output coincident with the vasodilatation, and hence mean arterial blood pressure changed little. In both Long Evans and Brattleboro rats in the water-replete state there was no indication of a negative effect of DuP 753 on cardiac haemodynamics. Thus, the

Table 2 Changes (relative to pre-drug baseline) in cardiovascular variables in water-replete ($n = 8$) and water-deprived ($n = 9$) Brattleboro rats 15 min after DuP 753 (10 mg kg⁻¹)

	Water-replete	Water-deprived
Heart rate (beats min ⁻¹)	32 ± 10*	58 ± 11*
Mean arterial blood pressure (mmHg)	-10 ± 4*	-39 ± 3*†
Cardiac output (ml min ⁻¹)	0 ± 4	5 ± 2
Peak aortic flow (ml min ⁻¹)	9 ± 9	39 ± 8*†
+ dF/dt _{max} (l min ⁻²)	36 ± 35	144 ± 56
Stroke volume (μl beat ⁻¹)	-26 ± 14	-28 ± 5*
Total peripheral conductance (ml min ⁻¹ mmHg ⁻¹)	148 ± 104	482 ± 36*†

Values are mean ± s.e. mean.

* $P < 0.05$ versus baseline.

† $P < 0.05$ water-deprived versus water-replete.

Figure 2 Cardiovascular variables in conscious, water-replete, Brattleboro rats ($n = 8$). At the arrows between 0 and 60 min, increasing i.v. bolus doses (0.1 – 10 mg kg^{-1}) of DuP 753 were administered (i.e. at 15 min intervals). Fifteen min after the highest dose of DuP 753 a supramaximal dose of captopril (2 mg kg^{-1}) was administered. The values shown are the means, and the bars represent s.e. means; where the bars are not visible they lie within the symbol. * $P < 0.05$ versus pre-injection value for that dose; † $P < 0.05$ versus original pre-drug baseline. For clarity this latter comparison was made only for the 15 min post-injection values. The dashed line represents the original baseline.

modest hypotensive effects of DuP 753, even at the highest dose employed, appeared to be entirely due to a rise in total peripheral conductance. It is likely the transient increases in cardiac output, peak aortic flow and $+dF/dt_{\max}$ were a consequence of this reduction in afterload, and possibly contributed to by reflex responses to the latter.

The vasodilator effect of DuP 753 in water-replete, Long Evans and Brattleboro rats indicates that endogenous AII is involved in the tonic control of total peripheral conductance in these animals, and our previous experiments indicate the renal and mesenteric vascular beds are important targets for this effect (Batin *et al.*, 1991). In the present study we were careful to ensure there was no post-operative hypovolaemia in the Long Evans rats; thus the results probably represent the normal condition of the animals studied. An involvement of endogenous AII in the tonic control of total peripheral conductance is consistent with our previous findings with captopril in water-replete Long Evans and Brattleboro rats (Gardiner & Bennett, 1985; Muller *et al.*, 1990). The present observation that a supramaximal dose of captopril was without an additional hypotensive effect after DuP 753 (10 mg kg^{-1}) had been administered indicates that the properties of captopril, other than its ability to inhibit AII generation, are not likely to have contributed to its acute hypotensive or vasodilator effects in previous experiments (Gardiner & Bennett, 1985; Tomlinson *et al.*, 1990; Muller *et al.*, 1990). We have no ready explanation for the small increases in heart rate, mean arterial blood pressure, peak aortic flow and $+dF/dt_{\max}$ seen following administration of captopril to water-replete Long Evans rats.

The lack of any signs of a negative inotropic effect of DuP 753 under conditions in which it caused clear vasodilatation

indicates that, in the water-replete state, endogenous AII was not exerting an important influence on cardiac function (Urata *et al.*, 1990; Hirakata *et al.*, 1990; Lindpaintner *et al.*, 1990). It is likely the gradual reduction in stroke volume following DuP 753 in the water-replete condition (most marked in Brattleboro rats) was due to the tachycardia causing a shortening of cardiac filling time.

Cardiac haemodynamic changes following DuP 753 in water-deprived Brattleboro rats

In water-deprived Brattleboro rats, in the resting state, there was a clear-cut reduction in cardiac output. However, mean arterial blood pressure was raised because there was a disproportionately large reduction in total peripheral conductance (Table 1). While these findings could indicate abnormality of baroreflex control in water-deprived Brattleboro rats, we have been unable to show impairment of baroreflex modulation of pulse interval under these conditions (Gardiner & Bennett, 1988).

The marked reduction in total peripheral conductance in water-deprived Brattleboro rats was abolished following administration of the highest dose of DuP 753 (10 mg kg^{-1}) and although there was a significant difference in the changes of total peripheral conductance in water-replete and water-deprived Brattleboro rats in this circumstance (Table 2), the absolute values of total vascular conductance were not different (Figures 2 and 3). However, given that DuP 753 can influence both the pre- and postjunctional actions of AII (Chiu *et al.*, 1990; Wong *et al.*, 1990a,b,c,d,e,f) it is not possible to determine from the present experiments the extent to which

Figure 3 Cardiovascular variables in conscious water-deprived Brattleboro rats ($n = 9$). At the arrows between 0 and 60 min, increasing i.v. bolus doses (0.1 – 10 mg kg^{-1}) of DuP 753 were administered (i.e. at 15 min intervals). Fifteen min after the highest dose of captopril (2 mg kg^{-1}) was administered. The values shown are the means, and the bars represent s.e. means; where the bars are not visible they lie within the symbol. * $P < 0.05$ versus pre-injection value for that dose; † $P < 0.05$ versus original pre-drug baseline. For clarity this latter comparison was made only for the 15 min post-injection values. The dashed line represents the original baseline.

inhibition of these two processes contributed to the vasodilator effects of DuP 753.

It is notable that even in water-deprived Brattleboro rats with marked activation of the RAS the hypotensive effects of DuP 753 were not contributed to by reductions in cardiac output. On the contrary, administration of DuP 753 caused increases, (albeit generally transient), in cardiac output, peak aortic flow and $+dF/dt_{\max}$. These changes went hand-in-hand with the increases in total peripheral conductance and hence, as in water-replete rats, were probably due to reflex changes and the reduction in afterload. Thus, even under conditions in which circulating levels of AII are known to be elevated chronically (Bennett & Gardiner, 1985) it did not appear that DuP 753 exerted cardiac effects that could be attributed to inhibition of a myocardial supportive action of endogenous AII (Urata *et al.*, 1990; Hirakata *et al.*, 1990; Lindpaintner *et al.*, 1990).

The absence of any additional hypotensive effects of captopril following administration of DuP 753 to water-deprived

Brattleboro rats indicates that the profound hypotensive and vasodilator effects of captopril, alone, in this model (Gardiner & Bennett, 1985; Tomlinson *et al.*, 1990; Muller *et al.*, 1990) can also be explained by inhibition of the generation of AII and, furthermore, its hypotensive effects do not seem to involve suppression of cardiac output in this setting. Indeed, it is notable that captopril (and DuP 753) caused tachycardia in water-deprived rats, as we have noted previously (Gardiner & Bennett, 1985; Tomlinson *et al.*, 1990; Muller *et al.*, 1990; Batin *et al.*, 1991). This finding is at odds with the frequent assertion that reduction of blood pressure by inhibitors of the RAS is not accompanied by tachycardia. However, it may be that the hypotensive effects of inhibitors of the RAS are accompanied by less tachycardia than would be seen with other hypotensive agents (Batin *et al.*, 1991).

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