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Haemodynamic effects of the selective phosphodiesterase 5 inhibitor, UK-357,903, in conscious SHR

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1 Regional haemodynamic responses to a continuous, 4-day infusion of the selective phosphodiesterase type 5 inhibitor, UK-357,903 (0.133 or 1.33 mg kg^{-1} h^{-1}) were measured in conscious spontaneously hypertensive rats, and compared with those of enalapril (1 mg kg^{-1} h^{-1}).

2 Both doses of UK-357,903 caused modest reductions in mean blood pressure that were not dose-dependent and only significantly different from the vehicle effects on Day 1 of the study (mean −11.8 and −15.3 mmHg for low and high doses, respectively). UK-357,903 had mesenteric and hindquarters vasodilator effects, which were, again, similar for both dose levels and only significantly different from vehicle on Day 1. Neither dose of UK-357,903 affected renal vascular conductance or heart rate.

3 Although the haemodynamic effects of UK-357,903 were not clearly dose-related and some appeared to wane with time, geometric mean plasma levels of UK-357,903 increased in proportion to dose, and were sustained throughout the infusion period. Furthermore, plasma cyclic guanosine monophosphate, a biomarker of phosphodiesterase 5 inhibition, was persistently elevated, and increased with increasing dose.

4 Enalapril caused a fall in mean blood pressure on day 1 (−14.1 mmHg) that was associated with dilatation in renal, mesenteric and hindquarters vascular beds. The haemodynamic effects of enalapril were sustained or increased over the 4-day infusion, although plasma free drug levels were stable.

5 In conclusion, we have shown regional and temporal changes in the haemodynamic effects of UK-357,903, which may be due to activation of compensatory mechanisms, but there were no signs of functional compensation to the cardiovascular effects of enalapril.
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Abbreviations: ACE, angiotensin-converting enzyme; cGMP, cyclic guanosine monophosphate; HDAS, haemodynamics data-acquisition system; LCMS, liquid chromatography-mass spectrometry; MCA, monochloroacetic acid; MRM, multiple reaction monitoring; NO, nitric oxide; PDE5, phosphodiesterase type 5; SHR, spontaneously hypertensive rat

Introduction

Nitric oxide (NO) is involved in several aspects of cardiovascular regulation, the most well known being its vascular smooth muscle-relaxing activity, mediated by activation of soluble guanylyl cyclase, leading to an increase in the production of cyclic guanosine monophosphate (cGMP) (Moncada *et al*., 1991). Phosphodiesterase type 5 (PDE5) is a cGMP-specific hydrolytic enzyme (see Rotella (2002) for review), and since PDE5 is the main PDE involved in the regulation of cGMP in vascular smooth muscle cells (Wallis *et al*., 1999), it would be expected that selective PDE5 inhibitors might have cardiovascular effects by augmenting the relaxant effects of endogenously produced, or administered, NO. Indeed, several studies have shown such effects, *in vitro* and *in vivo,* of the prototypic PDE5 inhibitor zaprinast (e.g., Harris *et al*., 1989; McMahon *et al*., 1989; Dundore *et al*., 1992; Merkel *et al*., 1992), which could be explained by its influence on cGMP. More recently, compounds with greater selectivity and potency than zaprinast have been developed, and their smooth muscle relaxant and blood pressure-lowering actions on acute administration have been demonstrated in experimental animals (e.g., Delpy & Le Monnier de Gouville, 1996).

Sildenafil is an orally active, highly selective, PDE5 inhibitor, which is successfully being used in the treatment of erectile dysfunction (see Rotella (2002) for review). The effectiveness of the drug in this condition is believed to be due to the inhibition of cGMP degradation in the corpus cavernosum and penile vasculature (where PDE5 is found in high concentrations (Boolell *et al*., 1996)), enhancing NO-mediated smooth muscle relaxation during sexual stimulation, and thus promoting penile erection.

Cardiovascular effects of sildenafil in healthy volunteers have been described (Jackson *et al*., 1999; Zusman *et al*., 1999; Vardi *et al*., 2002). For example, local intra-arterial

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administration of sildenafil into the forearm has been shown to cause arterial and venous dilatation, with some indication of preferential action on the venous circulation (Zusman *et al*., 1999). After oral dosing, sildenafil was shown to cause a modest (up to 10 mmHg) hypotension, which was not dose-dependent. The fall in blood pressure was associated with slight systemic vasodilatation, with no changes in heart rate or cardiac index (Jackson *et al*., 1999). Interestingly, the maximum effect of sildenafil on blood pressure in a small group of treated hypertensive patients appeared greater than that in normotensive volunteers, and was associated with a significant reduction in augmentation index, suggesting reduced vascular tone in the arteries (Mahmud *et al*., 2001). In contrast, other groups have reported that the effects on blood pressure of single doses of sildenafil together with commonly used antihypertensive medications are small and similar to those in untreated normotensive men (Zusman *et al*., 2000; Vardi *et al*., 2002), although in the study of Vardi *et al*. (2002) it was found that 22.7% of hypertensive men, but only 3.7% of normotensive men, experienced systolic BP reductions of 20 mmHg or greater without hypotensive symptoms. This may indicate that PDE5 inhibitors could have significant antihypertensive effects in a subset of hypertensive patients.

To our knowledge, the effects of a selective PDE5 inhibitor on blood pressure and regional haemodynamics under conditions where consistent exposure is maintained over a prolonged period have not been reported. Nevertheless, as sildenafil is beginning to be investigated in a chronic dose regimen, for example, to treat pulmonary hypertension (e.g., Sastry *et al*., 2002; Zimmermann *et al*., 2002), it is important to determine the haemodynamic effects of PDE5 inhibitors during continuous exposure. Therefore, the aim of the present study was to assess the blood pressure-lowering and regional haemodynamic effects of a highly selective PDE5 inhibitor, UK-357,903 (Table 1), when administered by continuous infusion over 4 days. Given the evidence that PDE5 inhibition may result in more marked haemodynamic effects in hypertensive patients (see above), our experiments were performed in conscious, spontaneously hypertensive rats (SHRs) (see also Delpy & Le Monnier de Gouville, 1996).

Angiotensin-converting enzyme (ACE) inhibitors are widely used as antihypertensives, and the cardiovascular effects of ACE inhibition have also been postulated to be, in part, due to enhancement of NO-mediated vasodilatation as a downstream response to increased bradykinin levels (Kohno *et al*., 1999; Squire *et al*., 2000). Some of the cardiovascular effects of ACE inhibition may, therefore, be attributable to enhancement of the endogenous NO-cGMP system Thus, the effects of UK-357,903 were compared with those of the ACE inhibitor, enalapril given at a dose which, on the basis of pilot studies, appeared to cause similar acute blood pressure-lowering effects to those of UK-357,903 in SHRs.

Methods

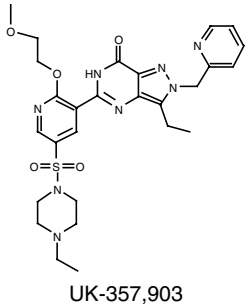
All experiments were carried out on male, SHRs (Charles River, U.K.), weighing between 280 and 350 g (i.e., between 20 and 22 weeks of age) at the time of study. Animals were housed in a temperature-controlled environment (20–22°C), with a 12 h light–dark cycle (lights on at 06:00 h), with free access to food and water throughout. The procedures were approved by the University of Nottingham Ethical Review Committee, and were performed under Home Office Project Licence authority.

Surgical preparation

Surgery was performed in two stages, under general anaesthesia (fentanyl and medetomidine, 300 $\mu g\,kg^{-1}$ of each, i.p). The first stage involved implantation of miniaturized, pulsed Doppler flow probes around the left renal artery, the superior mesenteric artery and the distal abdominal aorta (below the level of the ileocaecal artery, to monitor flow to the hindquarters). The second stage involved placement of catheters in the distal abdominal aorta (*via* the caudal artery) to monitor the arterial blood pressure and heart rate, and in the right jugular vein for drug administrations. After each surgical stage, anaesthesia was reversed, and analgesia provided with atipamezole and nalbuphine, respectively ($1\,mg\,kg^{-1}$ of each, s.c.). The two surgical stages were separated by at least 10 days and, prior to the second stage, the fitness of all animals was certified by the named veterinary surgeon.

After catheterization, double-channel, fluid-filled swivels (Blair *et al*., 1980) were used to allow overnight i.v. infusion of drugs or saline ($0.4\,ml\,h^{-1}$) and i.a. infusion of heparinized ($15\,U\,ml^{-1}$, $0.4\,ml\,h^{-1}$) saline to maintain catheter patency.

Table 1 Structure of UK-357,903 and IC_{50} values for inhibition of phosphodiesterase (PDE) enzymes



UK-357,903

Enzyme	*Geometric mean IC_{50}* (nM) (n ⩾ 3)	*95% CI*
PDE5	1.3	(1.1–1.6)
PDE6[a]	714	(575–887)
PDE1-3 and 7-11	> 10,000	—

[a]rod photoreceptor. Human PDE enzymes were prepared as previously described (Ballard *et al.,* 1998; Gbekor *et al*., 2002). IC_{50} values were determined using [^{3}H]-cGMP (PDE1, 2, 5, 6, 9, 10 and 11) or [3 H]-cAMP (PDE3, 4, 7 and 8) as substrate essentially as described by Fawcett *et al*. (2000).

Experiments began 24 h after catheterization, when the animals were fully conscious, freely moving, and with access to food and water *ad libitum*.

Cardiovascular recordings

Continuous recordings of cardiovascular variables were made using a customized, computer-based system (Haemodynamics Data Acquisition System (HDAS), University of Limburg, Maastricht) connected to the transducer amplifier (Gould model 13-4615-50) and the Doppler flowmeter (Crystal Biotech VF-1 mainframe (pulse repetition frequency 125 kHz) fitted with high-velocity (HVPD-20) modules). Raw data were sampled by HDAS every 2 ms, averaged every cardiac cycle, and stored to disc at 5 s intervals. Data were analysed offline using software (Datview, University of Limburg, Maastricht) which interfaced with HDAS.

Experimental protocol

After a control period of at least 90 min baseline recording on day 1, rats were randomized to receive UK-357,903 ($133\,\mu g\,kg^{-1}\,h^{-1}$ ($n=8$) or $1.33\,mg\,kg^{-1}\,h^{-1}$. ($n=8$)), enalapril ($1\,mg\,kg^{-1}\,h^{-1}$, $n=9$) or vehicle (isotonic saline adjusted to pH 4 with HCl, $n=8$), all infused at $0.4\,ml\,h^{-1}$. Thereafter, drugs or vehicle were administered continuously until the end of the experiment. Cardiovascular data were collected for 7 h after the onset of drug administration on day 1, and for periods of 7 h on days 2–4.

Arterial blood samples were collected into tubes containing EDTA (as anticoagulant and phosphodiesterase inhibitor) prior to any intervention on day 1 and after the recording period of each experimental day. Plasma was prepared and stored frozen at $-20^{\circ}C$ until analysed for drug levels and cGMP.

CGMP, renin activity and compound analyses

The concentration of cGMP in plasma was determined by enzyme immunoassay, using Kit RPN 226 from Amersham Pharmacia Biotech U.K. Ltd (Little Chalfont, Bucks, U.K.). In brief, 50 μl samples of plasma were diluted with 950 μl of kit assay buffer and taken through the acetylation protocol supplied with the kit. The recovery of standard amounts of cGMP ($25\,pmol\,ml^{-1}$) spiked into rat plasma was $160\pm16\%$ (mean $\pm$ s.e.m.) after correction for the endogenous level, suggesting that plasma enhanced the assay sensitivity. No adjustment was made for this apparent recovery.

Plasma renin activity was determined by monitoring the formation of angiotensin I from endogenous plasma angiotensinogen using the Angiotensin I [^{125}I] Radioimmunoassay Kit NEA104,105 (Perkin-Elmer Life Sciences, Inc. Boston, MA, U.S.A.). The procedure was as described in the kit protocol, except that volumes for the angiotensin I generation step were scaled down to accept 100 μl of plasma. Plasma concentrations of UK-357,903 and enalaprilat (active metabolite of enalapril rapidly formed in rat plasma) were determined by liquid chromatography-mass spectrometry (LC-MS), using a Sciex API2000 mass spectrometer (Perkin-Elmer Sciex, Foster City, CA, U.S.A.) operating in the positive ion mode using the TurboIonspray® interface. For UK-357,903 analysis, plasma samples (50–100 μl) were diluted with 1 ml 1 M monochloroacetic acid (MCA) dissolved in 90:10 H_2O: methanol, and 20 μl of a solution of a structurally related internal standard ($2\,\mu g\,ml^{-1}$: 40 ng) was added. UK-357,903 was extracted from plasma by a cation exchange method using Waters Oasis MCX 96-well extraction plates (Waters Corporation, MA, U.S.A.), washed (1 ml MCA, 1 ml water and 1 ml methanol, sequentially) and eluted with 1 ml 5% ammonia in acetonitrile. After evaporation to dryness under N_2, the residue was reconstituted in 300 μl 70:30 ($v\,v^{-1}$) methanol:-water containing 2 mM ammonium acetate. Samples (180 μl) were analysed by LC-MS/MS. Data were acquired in the multiple reaction monitoring (MRM) mode at a collision energy of 55 eV, using N_2 as collision gas. Chromatography was performed using a 5 μm Hypersil HS100 C18 column (50 mm × 4.6 mm i.d.) (Thermo Hypersil-Keystone, Runcorn, Cheshire, U.K.) and a mobile phase of 90:10 ($v\,v^{-1}$) methanol:-water containing 2 mM ammonium acetate, pH 4.0. Samples were eluted from the column at a flow rate of $1\,ml\,min^{-1}$, and the flow split post-column (50:1) before entering the mass spectrometer. Peaks were quantified from a calibration curve of extracted standards prepared in plasma (range 0.5–200 ng per sample) using linear regression with one/y^2 weighting. Total plasma concentrations of UK-357,903 were converted to free levels using a free fraction value of 0.12.

For enalaprilat analysis, plasma samples (50 μl) were diluted with 50 mM phosphate buffer (pH 3) dissolved in MilliQ water (Millipore Corporation, Bedford, MA, U.S.A.), and 20 μl of a solution of a structurally related internal standard ($5\,\mu g\,ml^{-1}$:100 ng) was added. Enalaprilat was extracted from plasma using the Waters Oasis HLB 96 well extraction plate (Waters Corporation, MA, U.S.A.), samples were washed (1 ml pH 3 phosphate buffer and 1 ml 2% glacial acetic acid (%$v\,v^{-1}$ in MilliQ Water) sequentially, and eluted using 1 ml methanol. After evaporation to dryness under N_2 (37°C), the residue was reconstituted in 300 μl 10:90 ($v\,v^{-1}$) methanol:-water containing 2 mM ammonium acetate, and any residual particulates were removed by centrifugation (3000 r.p.m., 10 min at 4°C) prior to injection. Samples (180 μl) were analysed by LC-MS-MS. Data were acquired in MRM mode at a collision energy of -30 mV using argon as the collision gas. Chromatography was performed using a Chromolith SpeedRod® RP –18e column (50 × 4.6 mm i.d) (Merck KGaA, Darmstadt, Germany) and mobile phases A (90:10 water:-methanol containing 2 mM ammonium acetate, pH 3.0) and B (90:10 ($V\,V^{-1}$) methanol:water containing 2 mM ammonium acetate, pH 4.0). A gradient of 0–0.5 min 100% A; 0.5–1 min changing to 100% B, 1–1.5 min 100% B and 1.51–2.2 min 100% A was used with a flow rate of $3\,ml\,min^{-1}$. The flow was split post-column (1:5) before entering the mass spectrometer. Peaks were quantified from a calibration curve of extracted samples prepared in plasma (range 0.2–200 ng per sample) using linear regression with $1/y$ weighting. Total plasma concentrations of enalaprilat were converted to free levels using a free fraction value of 0.90.

Cardiovascular data analysis

The study ran over several weeks and typically, in each week, data for equal numbers of rats from each treatment group were collected. Cardiovascular data were collected for 7 h after the onset of drug administration on day 1, and for periods of 7 h on days 2–4. The baseline was taken as the 30–45 min period

prior to drug administration on day 1, when the animals were settled. For graphical representation, recordings from each day were divided into four periods, that is, baseline then 3×140 min averages on day 1 and 4×105 min averages on days 2–4. For statistical analyses, postdosing data were averaged across the entire 7 h recording period on each day. For each day, mean heart rate, blood pressure and free plasma cGMP concentration for each animal were analysed using analysis of covariance (ANOCOVA), allowing for potential week-to-week differences, and for differences at baseline. Similarly, analysis of % change in Doppler shift, % change in conductance and free plasma UK-357,903 concentration data was performed for each day using analysis of variance (ANOVA), again allowing for potential week-to-week differences. All concentration data were analysed on the log scale. The repeated measures were summarized using an average response for each animal on each day. Then ANOVA or ANOCOVA suitable for a randomized block design (where week is the block) was performed on the averaged data for each day. The comparisons of interest were the differences amongst the estimated treatment means on each day. As we took summary measures, no correction for the repeated measures was used.

The estimated treatment differences presented reflect the differences between each treated group and the vehicle group on each day. These estimates arise naturally from these methods of analysis and compensate for differences at baseline and week-to-week differences; 95% confidence intervals are presented with the estimated differences and these show the range of values within which the true treatment differences are likely to lie. For the cGMP data, the comparisons of the treatment groups are represented by treatment ratios, reflecting ratios of estimated means. Here a ratio of 1 indicates no difference between treatment groups.

Drugs

Fentanyl citrate was from Janssen-Cilay (High Wycombe U.K.); medetomidine hydrochloride (Domitor) and atipamezole hydrochloride (Antisedan) were from Pfizer (Kent, U.K.), nalbuphine hydrochloride (Nubain) was from Bristol-Myers Squibb (Hounslow, U.K.). The PDE5 inhibitor, 1-ethyl-4-{3-[3-ethyl-6,7-dihydro-7-oxo-2-(2-pyridylmethyl)-2*H*-pyrazolo 4,3-d]pyrimidin-5-yl]-2-(2-methoxyethoxy)-5-pyridylsulphonyl} piperazine (UK-357,903; Table 1), was supplied by Pfizer U.K., and enalapril was purchased from Sigma, U.K. Drugs and vehicle were infused at a rate of 0.4 ml h^{-1}.

Results

Cardiovascular responses

Resting cardiovascular variables in the four groups of rats prior to drug or vehicle administration were not significantly different (Table 2).

Figures 1–3 show plots of mean blood pressure plus heart rate (Figure 1), Doppler shift (Figure 2) and vascular conductance (Figure 3), respectively, together with estimated treatment effects (i.e., adjusted mean differences from vehicle) on each of the four study days.

On day 1, there were similar, significant, falls in mean blood pressure in rats treated with either dose of UK-357,903 or with enalapril, but no change in heart rate (Figure 1). In the enalapril-treated group only, there was an increase in renal Doppler shift (Figure 2) and vascular conductance, but in all the three treatment groups there were increases in mesenteric and hindquarters vascular conductances (Figure 3). In the group treated with the low dose of UK-357,903, the hindquarters vasodilatation was associated with a significant increase in Doppler shift (Figure 2).

On days 2–4, the effects of UK-357,903 on blood pressure and hindquarters vascular conductance appeared to decline relative to day 1, and were not significantly different from the vehicle (Figures 1 and 3). The mesenteric vasodilator effect of UK-357,903 appeared to be maintained, but due to increased variability in the data, relative to day 1, was not significantly different from vehicle (Figure 3). The blood pressure-lowering and widespread vasodilator effects of enalapril were consistently greater than those of UK-357,903 on days 2–4, and were maintained or showed a trend to increase (Figures 1 and 3).

Plasma drug levels

Plasma concentrations of free UK-357,903 were dose-related and relatively stable across the four experimental days, and plasma concentrations of enalaprilat were consistently elevated (Table 3). The free levels of UK-357,903 were approximately 2.5–3.5-fold (low dose) and 24–33-fold (high dose) the IC_{50} for inhibition of PDE5 (Table 1).

Plasma cGMP concentrations and renin activity

UK-357,903 caused dose-dependent rises in plasma cGMP to approximately two and four-fold vehicle level for the low and high doses, respectively, which persisted across the four experimental days. In contrast, enalapril was without

Table 2 Resting cardiovascular variables in conscious spontaneously hypertensive rats

Treatment group	*UK 357,903* (133 μg kg^{-1} h^{-1})	*UK-357,903* (1.33 mg kg^{-1} h^{-1})	*Enalapril*	*Vehicle*
Heart rate (beats min^{-1})	314±7	308±10	307±8	308±8
Mean BP (mmHg)	159±5	158±7	168±3	154±3
Renal Doppler shift (kHz)	9.0±1.1	5.7±0.6	6.9±0.4	7.3±0.7
Mesenteric Doppler shift (kHz)	9.0±0.8	9.2±0.8	8.7±0.6	8.9±1.1
Hindquarters Doppler shift (kHz)	4.3±0.3	3.7±0.2	3.7±0.2	3.8±0.3
Renal conductance (units)	57±7	37±5	41±2	47±4
Mesenteric conductance (units)	57±6	59±6	52±4	57±7
Hindquarters conductance (units)	27±2	24±2	22±2	25±2

Values are mean±s.e.m. for groups of eight or nine rats. Units for vascular conductance are (kHz $mmHg^{-1}$) $\times 10^3$.

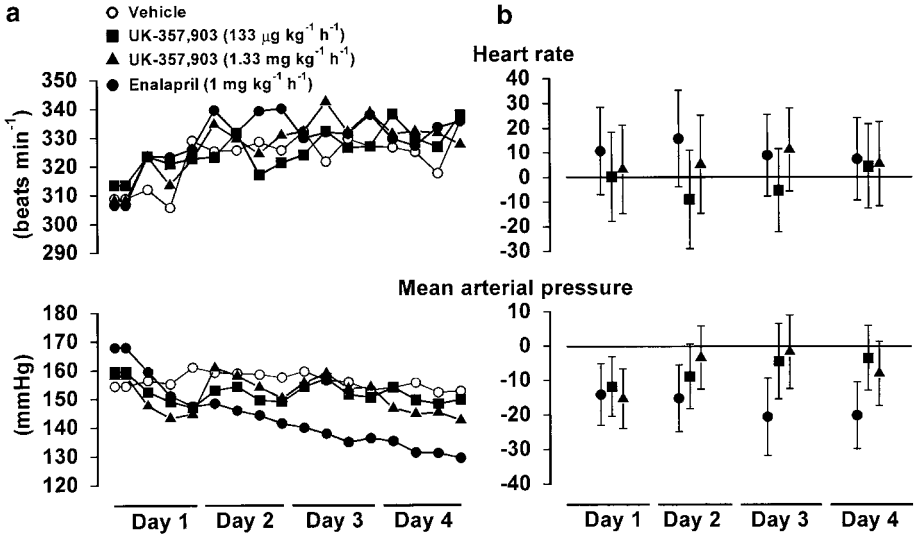


Figure 1 Heart rate and mean arterial blood pressure over a 4-day continuous infusion of vehicle ($n=8$), UK-357,903 ($133\,\mu g\,kg^{-1}\,h^{-1}$; $n=8$), UK-357,903 ($1.33\,mg\,kg^{-1}\,h^{-1}$; $n=8$) or enalapril ($1\,mg\,kg^{-1}\,h^{-1}$; $n=9$). Panel (a) shows values averaged over 105 min epochs during the 7 h monitoring period on each day. Panel (b) shows the estimated differences between each treatment group and vehicle with 95% confidence intervals. The estimated baseline values for blood pressure and heart rate were 160.2 mmHg and 309 beats min^{-1}, respectively. Treatment effects are significantly different from vehicle ($P<0.05$), where the confidence interval bar does not cross the zero line.

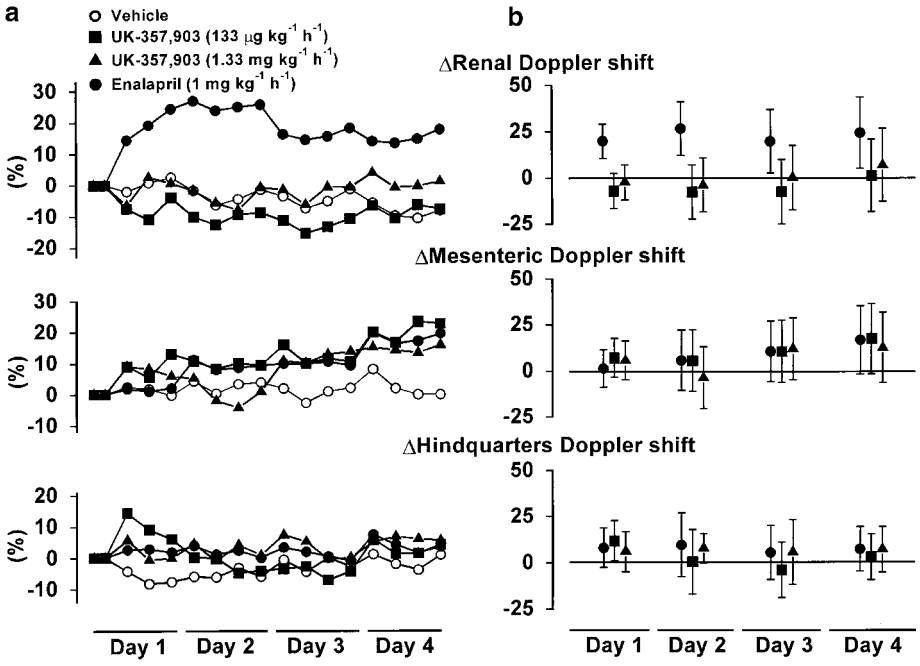


Figure 2 Changes in regional Doppler shift over a 4-day continuous infusion of vehicle ($n=8$), UK-357,903 ($133\,\mu g\,kg^{-1}\,h^{-1}$; $n=8$), UK-357,903 ($1.33\,mg\,kg^{-1}\,h^{-1}$; $n=8$) or enalapril ($1\,mg\,kg^{-1}\,h^{-1}$; $n=9$). Panel (a) shows values averaged over 105 min epochs during the 7 h monitoring period on each day. Panel (b) shows the estimated differences between each treatment group and vehicle with 95% confidence intervals. Treatment effects are significantly different from vehicle ($P<0.05$), where the confidence interval bar does not cross the zero line.

significant effect, except on day 3 where there was a small increase to approximately 1.4-fold the vehicle level (Figure 4).

Plasma renin activity levels (geometric mean in $ng\,ml^{-1}\,h^{-1}$ with 95% CI) determined on day 2 of the study were similar in the vehicle, and the low and high dose UK-357,903 treatment groups; 2.2 (1.4, 3.5; $n=8$), 2.9 (1.8, 4.7; $n=8$), and 3.1 (2.0, 4.9, $n=8$), respectively. In contrast, renin activity in the enalapril group, 18.0 (11.1, 29.1, $n=8$), was significantly increased relative to the vehicle group ($P<0.001$).

Discussion

The aim of the study was to assess the regional haemodynamic effects of continuous, 4-day infusion of the selective PDE5 inhibitor, UK-357,903, in conscious SHR, and to compare them with those of the angiotensin converting-enzyme inhibitor enalapril.

The results clearly show that UK-357,903 caused significant, but modest hypotension and regionally selective dilatation of

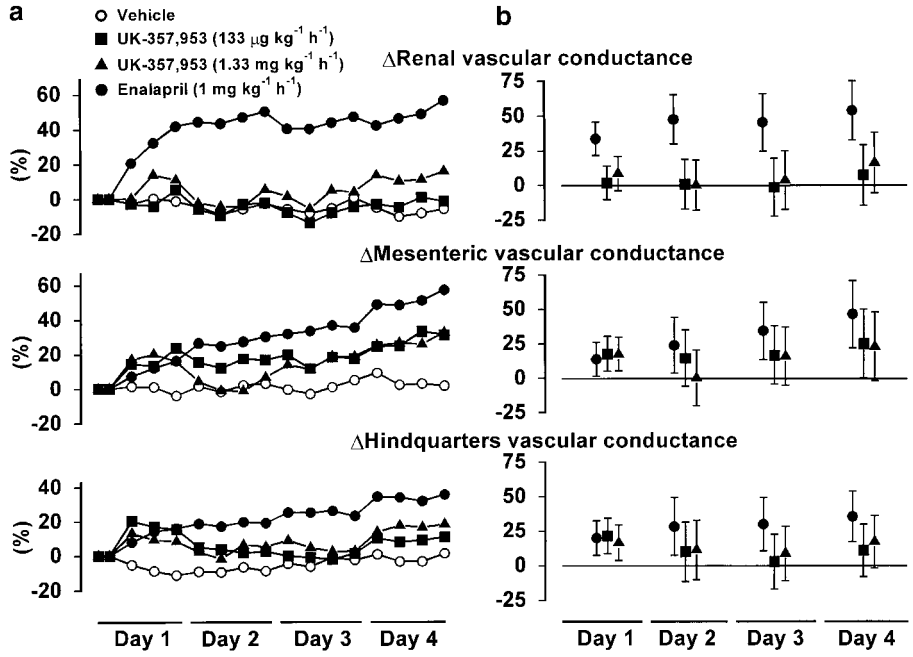


Figure 3 Changes in regional vascular conductance over a 4-day continuous infusion of vehicle ($n=8$), UK-357,903 ($133\,\mu g\,kg^{-1}\,h^{-1}$; $n=8$), UK-357,903 ($1.33\,mg\,kg^{-1}\,h^{-1}$; $n=8$) or enalapril ($1\,mg\,kg^{-1}\,h^{-1}$; $n=9$). Panel (a) shows values averaged over 105 min epochs during the 7 h monitoring period on each day. Panel (b) shows the estimated differences between each treatment group and vehicle with 95% confidence intervals. Treatment effects are significantly different from vehicle ($P<0.05$), where the confidence interval bar does not cross the zero line.

Table 3 Plasma concentrations of free UK-357,903 or enalaprilat

Treatment group	*UK-357,903* ($133\,\mu g\,kg^{-1}\,h^{-1}$)	*UK-357,903* ($1.33\,mg\,kg^{-1}\,h^{-1}$)	*Enalapril*
Day 1	4.6 [3.9, 5.3]	43 [36, 52]	2544 [2150, 3010]
Day 2	4.4 [3.8, 5.1]	39 [31, 47]	2467 [1811, 3360]
Day 3	4.4 [3.5, 5.6]	37 [29, 48]	2768 [2411, 3178]
Day 4	3.3 [2.2, 4.8]	31 [20, 48]	2358 [2015, 2759]

Values are geometric mean (nM) with [confidence intervals].

mesenteric and hindquarters vascular beds on the first day of administration. The hypotensive and hindquarters vasodilator effects appeared to decline on subsequent days and, although the mesenteric vasodilatation was generally of similar magnitude throughout the four study days, it was also only statistically significant on day 1. Neither dose level of UK-357,903 affected renal vascular conductance. In comparison, enalapril caused hypotension and vasodilatation in all monitored vascular beds, including renal, and its effects were maintained or showed a trend to increase during the study.

Interestingly, the cardiovascular effects of UK-357,903 not only declined after day 1, but also were not dose-related, and yet its effects on plasma cGMP levels were sustained and increased with increasing dose. Thus, the waning and lack of dose dependence in the haemodynamic changes may have been due to physiological antagonism of the effects of raised cGMP levels, or indicate that near maximal cardiovascular responses were achieved by submaximal inhibition of PDE5. Alternatively, although a likely source of cGMP in plasma during PDE5 inhibition is the vascular smooth muscle, it may also arise from platelets that are rich in PDE5. Hence, changes in

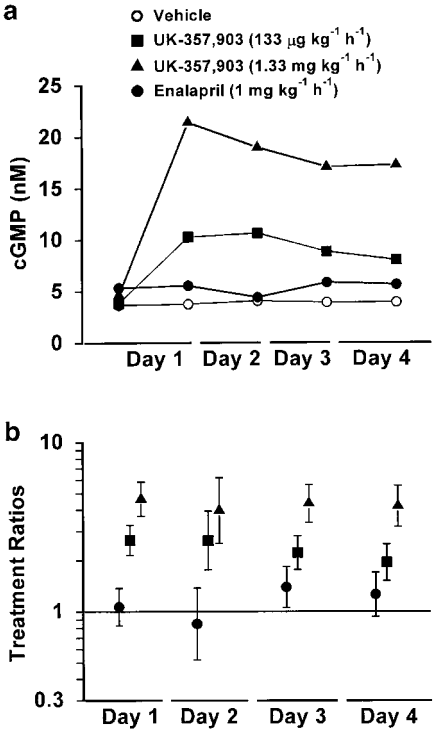


Figure 4 Plasma concentrations of cGMP during continuous infusion of vehicle ($n=8$), UK-357,903 ($133\,\mu g\,kg^{-1}\,h^{-1}$; $n=8$), UK-357,903 ($1.33\,mg\,kg^{-1}\,h^{-1}$; $n=8$) or enalapril ($1\,mg\,kg^{-1}\,h^{-1}$; $n=9$) in conscious SHR. Panel (a) shows the geometric mean concentrations of cGMP in plasma samples taken at the end of each days monitoring. Panel (b) shows the estimated treatment ratios, relative to vehicle, with 95% confidence intervals. Treatment effects are significantly different from vehicle ($P<0.05$), where the treatment ratio confidence interval does not cross the unity line.

the plasma level of cGMP during PDE5 inhibition may not prove to be directly correlated with vasodilatation.

Studies in man using sildenafil have also found effects on BP and systemic vascular resistance that were not dose-related (Jackson *et al*., 1999). Likewise, in anaesthetised dogs, Hosogai *et al*. (2001) showed no dose dependence in the blood pressure-lowering effects of another PDE5 inhibitor, FR 226807; however, that study did show dose-related hypotension in response to sildenafil. The explanation for the apparent difference between the effects of the two different PDE5 inhibitors was not obvious, although Hosogai *et al*. (2001) suggested that FR 226807 was, perhaps, more selective for PDE5.

There is evidence that sildenafil can cause sympathetic activation in man (Phillips *et al*., 2000) which, it has been suggested, was not secondary to arterial or cardiopulmonary baroreflex activation, and which may be selective for the vasculature, inasmuch as there was no accompanying tachycardia (Phillips *et al*., 2000). We also observed no persistent tachycardia following administration of UK-357,903, although there was a very transient rise in heart rate for the first 30 min following administration (data not shown). Hence, it is possible that, in the present study, UK-357,903-induced sympathetic activation, directed to the vasculature, served to offset a greater vasodilator response to the higher dose of UK-357,903, and may also have accounted for the lesser effect on blood pressure after day 1. Delpy & Le Monnier de Gouville (1996) have shown acute hypotensive effects of PDE5 inhibition in SHR, together with reductions in the pressor response to α-adrenoceptor agonists, and have suggested that a reduction in vasoconstrictor sensitivity may contribute to the blood pressure-lowering effects of PDE5 inhibitors. However, if this mechanism contributed to the haemodynamic effects that occurred in our experiments with UK-357,903, then it is feasible the effect would wane if reflex sympathetic activation occurred with chronic administration of the drug.

Sildenafil has recently been shown to prevent the circadian fall in plasma renin activity, and this has been taken as evidence for a stimulatory effect of the drug on renin secretion (Chiu & Reid, 2002). Whether or not this apparent effect of sildenafil is attributable to the increased cGMP or secondary to sympathetic activation (see above) is unclear. Whatever the mechanism, it is also feasible that activation of the renin-angiotensin system may have contributed to the decline in the vasodilator effects of UK-357,903 in the present study (see below). An analysis of plasma renin activity in samples taken at the end of day 2 in the present study showed no significant effect of either dose of UK-357,903 but an approximately eight-fold increase with enalapril. However, this does not preclude a functional involvement of the renin–angiotensin system.

The regional heterogeneity in the effects of UK-357,903 was notable, particularly the lack of effect of UK-357,903 on renal vascular conductance, because this was in striking contrast to the effects of enalapril. There is evidence to suggest that the β_2 subunit of soluble guanylyl cyclase is preferentially expressed in rat kidney, and can inhibit NO-mediated activation of the α_1/β_1 heterodimeric form of the enzyme, and thereby reduce NO-stimulated cGMP levels (Gupta *et al*., 1997). This could explain why the renal vasculature is less sensitive than other vascular beds to the vasodilator effects of the NO-cGMP system (Phillips *et al*., 1991), and could account for the lesser renal vasodilator effects of PDE5 inhibition. Furthermore, it has been suggested that increased expression of the β_2 subunit in Dahl salt-sensitive rats (Gupta *et al*., 1997) may explain the impaired renal vasodilator response to nitrovasodilators, coupled with impaired renal cGMP generation, in those animals (Simchon *et al*., 1996). Whether or not SHRs show a similar change in soluble guanylyl cyclase subunit expression is not known. However, since earlier acute studies using zaprinast in normotensive rats also found a relative resistance of the renal vascular bed to dilate (Trapani *et al*., 1991; Dundore *et al*., 1992), it seems unlikely that the regional heterogeneity observed here was unique to the SHR.

In our experience, albeit in normotensive rats, nitrovasodilators cause mesenteric, but not renal, vasodilatation unless the renin–angiotensin system is blocked, in which case the mesenteric vasodilator effect is enhanced and renal vasodilatation is unmasked (Phillips *et al*., 1991). Therefore, it is possible that, for the reasons outlined above, there is less tendency for the kidney than the mesentery to vasodilate in response to NO-cGMP activation and that, in the presence of renin–angiotensin system blockade, mesenteric vasodilator effects of UK-357,903 might be enhanced and renal vasodilatation might be revealed.

In marked contrast to UK-357,903, enalapril caused hypotension and vasodilatation that progressed across the 4 days of the experiment. Moreover, the vasodilator effect of enalapril was particularly marked in the renal vascular bed, where it was associated with hyperaemia. Others have reported preferential renal vasodilator effects of ACE inhibitors (Kanagawa *et al*., 1997) and angiotensin receptor antagonists (Li & Widdop, 1996) administered over a relatively short period to SHR.

The progressive haemodynamic effects of enalapril were against the background of stable plasma drug levels, although it would be expected that chronic dosing with an angiotensin converting-enzyme inhibitor would have natriuretic and diuretic effects in addition to causing vasodilatation, and these effects could contribute to the progressive fall in mean BP. However, it was clear from our results that progressive vasodilatations contributed to the incremental hypotension. Increasing antihypertensive effects of ACE inhibitors and angiotensin receptor blockers have also been noted previously when the drugs were administered daily over a period of 5 days (Oosting *et al*., 1999). Those authors suggested possible explanations for this were pharmacokinetic, that is, incremental tissue penetration and accumulation of the drugs and their metabolites, and pharmacodynamic, that is, increasing dependency on the renin–angiotensin system as a result of the natriuretic actions of the drugs (Oosting *et al*., 1999). Other possible explanations might include changes in endothelium-dependent vasodilatation (Kähönen *et al*., 1999; Feng *et al*., 2000), increased NO synthase expression (Qadri *et al*., 2001), and/or reduced sensitivity to the vasoconstrictor effects of endothelin (Miki *et al*., 2002), all of which have been shown following relatively short-term treatment with angiotensin-converting enzyme inhibitors in SHR. Whatever the explanation, it is interesting to note that these results differ from those we previously obtained in another hypertensive rat strain (transgenic mRen-2 rats (Mullins *et al*., 1990)), inasmuch as the vasodilator effects of enalaprilat (given at a higher dose than the dose of enalapril used here) did not progress between 8 and 32 h of infusion; indeed the antihypertensive effect

diminished slightly, due to an increase in cardiac index (Gardiner *et al*., 1997).

In summary, we have demonstrated that the haemodynamic responses to a 4-day continuous infusion of the PDE5 inhibitor UK-357,903 show regional and temporal heterogeneity. It remains to be determined whether or not similar cardiovascular profiles would be seen with structurally different PDE5 inhibitors. Furthermore, whether or not the lack of renal vasodilator action of UK-357,903 and the transience of its antihypertensive actions are attributable to activation of counter-regulatory systems requires further investigation.

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