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Effects of endothelin ET_A receptor antagonism with PD 156707 on hemodynamics and renal vascular resistance in rabbits

Diane P. Ignasiak [a,*], Thomas B. McClanahan [a], Lori J. Saganek [a], Ronald E. Potoczak [a], Hussein Hallak [a], Kim P. Gallagher [a]

[a] *Vascular and Cardiac Diseases Section, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor, MI 48105, USA*

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Abstract

The objective of this study was to determine the in vivo effectiveness of the selective endothelin ET_A receptor antagonist PD 156707 {sodium 2-benzo[1,3]dioxol-5-yl-4-(4-methoxy-phenyl)-4-oxo-3-(3,4,5-trimethoxy-benzyl)-but-2-enoate}. Effectiveness was defined by the ability of the compound to block increases in renal vascular resistance and mean arterial blood pressure induced by an intravenous bolus of 0.3 nmol/kg of human endothelin-1 in pentobarbital anesthetized rabbits. Different groups of rabbits received hour long intravenous infusions of PD 156707 at doses of 0.003, 0.01, 0.03 or 0.3 mg/kg per h. During baseline conditions, mean arterial blood pressure, heart rate, renal blood flow, and renal vascular resistance were similar among the groups. The intravenous bolus of endothelin-1 significantly decreased mean arterial blood pressure (82 ± 3 mmHg to 65 ± 3 mmHg, $P < 0.05$) and increased renal vascular resistance (2.8 ± 0.3 mmHg/ml per min to 9.2 ± 1.1 mmHg/ml per min, $P < 0.05$) in untreated control animals. At doses of 0.3 and 0.03 mg/kg per h, PD 156707 virtually abolished endothelin-1 induced increases in renal vascular resistance, but did not affect the endothelin-1 induced decrease in mean arterial blood pressure. At 0.01 and 0.003 mg/kg per h, PD 156707 also inhibited endothelin-1 induced increases in renal vascular resistance but the effects were less striking, leading to the conclusion that the minimum effective intravenous dose of the compound in rabbits is in the range of 0.01–0.03 mg/kg per h. The results of this study demonstrate that PD 156707 is an extremely potent and highly selective endothelin ET_A receptor antagonist. In addition, this study demonstrates the utility of renal vascular resistance as an in vivo bioassay for evaluating the selective vascular effects of endothelin receptor antagonists in this species.

Keywords: Endothelin-1 ; Vascular resistance, renal; Endothelin ET_A receptor antagonist

1. Introduction

Endothelin-1 is a potent vasoconstrictor with a wide range of cardiovascular effects (Yanagisawa et al., 1988; Inoue et al., 1989). Elevated levels of plasma endothelin-1 have been reported in a variety of pathological conditions such as arterial hypertension (Saito et al., 1990), acute myocardial infarction (Kusumoto et al., 1993), congestive heart failure, and chronic renal disease (Koyama et al., 1989; Benigni et al., 1993). Although the specific significance of elevated endothelin-1 levels remains to be determined, blocking the effects of endothelin-1 with selective antagonists may be a potentially valuable therapeutic intervention (Benigni et al., 1993; Grover et al., 1993; Lee et al., 1994).

Two major subtypes of endothelin receptors have been identified and characterized. The vasoconstrictor effects of endothelin-1 appear to be primarily mediated by the endothelin ET_A receptor, while the endothelin ET_B receptor may mediate either vasodilation or vasoconstriction (Clozel et al., 1992; Sumner et al., 1992; Seo et al., 1994). The compound PD 156707 is a recently developed, selective, nonpeptide, endothelin ET_A receptor antagonist (Doherty et al., 1995). Reynolds et al. (1995a,b) showed that PD 156707 is 1300-fold more selective for the endothelin ET_A receptor than the endothelin ET_B receptor in rabbit tissue, but most in vivo pharmacologic work on this compound has been conducted in the rat, a species in which PD 156707 is much less selective.

Accordingly, the purpose of this study was to determine the effectiveness of PD 156707 in terms of endothelin ET_A receptor antagonism in the intact rabbit. Endothelin-1 is a potent vasoconstrictor in the renal vascular bed of the

* Corresponding author. Tel.: (1-313) 996-4364; Fax: (1-313) 996-1480; e-mail: ignasid@aa.wl.com

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rabbit (Rae et al., 1989), so renal vascular resistance was the main parameter used to determine the effectiveness of endothelin ET_A receptor blockade.

2. Materials and methods

2.1. Surgical procedure

Male New Zealand White (Hazelton) rabbits weighing 2–3 kg were anesthetized with 35 mg/kg intravenous sodium pentobarbital. Additional increments of pentobarbital in 0.2 ml doses were administered as needed throughout the experiment. A tracheostomy was performed and the animal was ventilated with room air supplemented with oxygen (Harvard Ventilator, Model 683). Arterial blood gasses were measured periodically to verify that PO_2 exceeded 100 mmHg and that PCO_2 and pH were within normal limits (Radiometer ABL510, Copenhagen, Denmark). Circulating water heating blankets were adjusted to maintain the rabbits at a normothermic temperature of approximately 39°C, which was measured with a rectal thermometer (Bailey Instruments, Saddlebrook, NJ, USA). The left femoral artery was cannulated with a polyethylene catheter which was used for continuous measurement of arterial blood pressure using a Statham P23Db transducer. The left femoral vein was cannulated for administration of PD 156707 and maintenance doses of pentobarbital. The left renal artery was exposed via a flank incision and a 1.5 mm ultrasonic flow probe (Transonic Flow Meter, Model T206, Ithaca, NY, USA) was placed around the vessel for measurement of renal blood flow.

2.2. Experimental protocol

Animals were randomized to receive either PD 156707 or a saline infusion. After the surgical instrumentation was complete, animals were allowed to stabilize for approximately 20 min at which point baseline measurements were obtained. Animals in the treatment groups randomly received one of the following doses of PD 156707: 0.003 mg/kg per h, 0.01 mg/kg per h, 0.03 mg/kg per h, or 0.3 mg/kg per h. All doses were infused in 10 ml of saline over a 1 h period. Control animals received an infusion of saline at 10 ml/h for 1 h. A second set of measurements was obtained to evaluate the effects of PD 156707, if any, on hemodynamics 1 h after starting the infusion of the compound (Post-Rx). A bolus dose of 0.3 nmol/kg of human endothelin-1 (Reynolds et al., 1995a) was administered intravenously at the end of the 1 h infusion of saline or PD 156707. The effects of this endothelin-1 'challenge' were compared to the Post-Rx measurements to determine the effectiveness of endothelin ET_A receptor blockade.

2.3. Plasma concentration assay

Heparinized blood samples were collected at baseline, at the end of the PD 156707 infusion (Post-Rx), and 3 min after the endothelin-1 bolus injection. Blood samples were centrifuged at 3000 rpm at 3°C for 10 min. The plasma was harvested and stored in a −70°C freezer for subsequent analysis of PD 156707 levels. Concentrations were determined by high-performance liquid chromatographic (HPLC) analysis (Rossi et al., 1996).

2.4. Drugs

PD 156707, a {sodium 2-benzo[1,3]dioxol-5-yl-4-(4-methoxy-phenyl)-4-oxo-3-(3,4,5-trimethoxy-benzyl)-but-2-enoate}, was synthesized by chemists at Parke-Davis as a sodium salt (Reynolds et al., 1995a; Doherty et al., 1995). The compound was dissolved in saline with all doses given in a 10 ml volume. Endothelin-1 (Peptide Institute, Osaka, Japan) was administered intravenously at 0.3 nmol/kg as a 1 ml bolus injection, brought to volume with saline.

2.5. Data analysis

Recordings were obtained during each experiment on a Gould eight-channel recorder and the analog signals were digitized on a Po-Ne-Mah digital acquisition system. Variables analyzed were heart rate, mean arterial blood pressure, renal blood flow, and renal vascular resistance (calculated as the quotient of mean arterial blood pressure divided by renal blood flow). Results are reported as mean values ± S.E.M. Statistical comparisons were made with analysis of variance followed by a Dunnett's test. For comparisons of hemodynamic data and renal vascular resistance within groups across time, repeated measures analysis of variance was used. When the time effect of the repeated measures analysis was significant, paired *t*-tests were used for multiple comparisons within each group. An overall α-level of 0.05 was considered to be statistically significant.

3. Results

3.1. General

A total of 30 rabbits were used in this study. Three rabbits in the control group were excluded because of unstable or low baseline renal blood flows (< 20 ml/min) and/or low mean arterial blood pressures (< 70 mmHg). Therefore, a total of 27 rabbits divided into five groups were used for data analysis in the study. Group 1 ($n = 10$) was the control group and received a total infusion of 10 ml/h of saline vehicle. Group 2 through group 5 received

Table 1
Data on mean arterial blood pressure and renal blood flow

	Baseline	Post-Rx	ET-1 challenge (maximum reduction)
Mean arterial blood pressure (mmHg)			
Vehicle	89 ± 3	82 ± 3 [b]	−19 ± 3 [c]
0.003 mg/kg	80 ± 3	75 ± 1	−13 ± 8
0.01 mg/kg	86 ± 4	79 ± 4	−26 ± 3 [c]
0.03 mg/kg	77 ± 3	76 ± 4	−24 ± 1 [c]
0.3 mg/kg	74 ± 5 [a]	66 ± 3 [a]	−19 ± 3 [c]
Renal blood flow (ml / min)			
Vehicle	32.3 ± 2.6	32.5 ± 3.1	−23.8 ± 3.5 [c]
0.003 mg/kg	30.4 ± 3.6	36.3 ± 4.5	−21.0 ± 3.1 [c]
0.01 mg/kg	30.0 ± 1.2	41.9 ± 6.1	−23.7 ± 4.2 [c]
0.03 mg/kg	41.9 ± 4.9	39.4 ± 3.6	−13.4 ± 0.9 [c]
0.3 mg/kg	31.1 ± 3.8	30.7 ± 3.1	−6.2 ± 4.3 [a]

Data are expressed as means ± S.E.M. [a] $P < 0.05$ compared to vehicle. [b] $P < 0.05$ compared to baseline. [c] $P < 0.05$ compared to Post-Rx. Abbreviations: baseline, before PD 156707 infusion; Post-Rx, at the end of the PD 156707 infusion and before the endothelin-1 challenge. Each of these time points represents a 5-min average. The column designated 'ET-1 challenge' represents the average maximal reduction in mean arterial blood pressure and renal blood flow within a 5-min period after the bolus challenge.

PD 156707 in 10 ml volumes: 0.003 mg/kg per h ($n = 4$), 0.01 mg/kg per h ($n = 5$), 0.03 mg/kg per h ($n = 4$) and 0.3 mg/kg per h ($n = 4$), respectively.

3.2. Plasma concentrations of PD 156707

The limit of quantitation for PD 156707 HPLC plasma assay was 10 ng/ml. Detectable plasma concentrations were evident at doses of 0.03 mg/kg per h and 0.3 mg/kg per h PD 156707. At 0.3 mg/kg per h, the plasma level of PD 156707 at the end of infusion averaged 200 ± 42.6 ng/ml. The plasma concentration at 0.03 mg/kg per h averaged 27.0 ± 5.8 ng/ml. At doses of 0.01 and 0.003 mg/kg per h, plasma concentrations were not detectable.

3.3. Hemodynamics

Hemodynamic data are summarized in Tables 1 and 2. During baseline conditions, before administration of PD 156707, mean arterial blood pressure and renal blood flow (Table 1) were similar among all five groups. After 1 h of intravenous administration of PD 156707, mean arterial blood pressure was significantly lower only in the high dose group (0.3 mg/kg per h) compared to vehicle. It should be noted, however, that the 0.3 mg/kg per h group had a lower mean arterial blood pressure during baseline conditions as well. When the trend for a reduction was compared to this group's own baseline value, the change was not statistically significant. There were no other significant differences in arterial blood pressure detected, suggesting that PD 156707 had minimal effects on arterial blood pressure in this model at the doses we used. There were no significant differences in renal blood flow among groups or within groups before or after administration of PD 156707 or vehicle.

Table 2
Data on renal vascular resistance

	Baseline	Post-Rx	ET-1 challenge (maximum increase)
Renal vascular resistance (mmHg / ml per min)			
Vehicle	3.1 ± 0.5	2.8 ± 0.3	6.4 ± 1.0 [b]
0.003 mg/kg	2.7 ± 0.3	2.2 ± 0.3	2.2 ± 0.6 [ab]
0.01 mg/kg	2.9 ± 0.2	2.0 ± 0.4	1.9 ± 0.7 [a]
0.03 mg/kg	1.9 ± 0.2	2.0 ± 0.1	0.5 ± 0.1 [ab]
0.3 mg/kg	2.4 ± 0.3	2.2 ± 0.3	0.4 ± 0.3 [a]

Data are expressed as means ± S.E.M. [a] $P < 0.05$ compared to vehicle. [b] $P < 0.05$ compared to Post-Rx. Abbreviations: baseline, before PD 156707 infusion; Post-Rx, at the end of the PD 156707 infusion and before the endothelin-1 challenge. Each of these time points represents a 5-min average. The column designated 'ET-1 challenge' represents the average maximal increase in renal vascular resistance occurring within a 5-min period after the endothelin-1 challenge.

Table 2 summarizes data on renal vascular resistance. There were no differences in renal vascular resistance across the groups during baseline conditions.

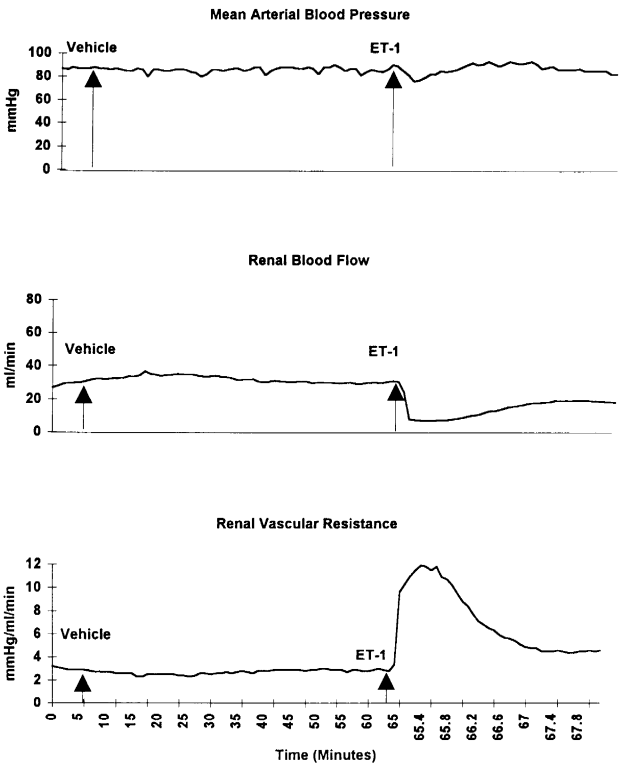

Fig. 1. A typical example of mean arterial blood pressure, renal blood flow, and renal vascular resistance over time in a control (vehicle) experiment. The arrows indicate when vehicle and endothelin-1 (ET-1) were given. Note the modest reduction in mean arterial blood pressure, large decrease in renal blood flow and the large increase in renal vascular resistance after the injection of endothelin-1.

3.4. Blockade of endothelin-1 challenge with PD 156707

After completion of the 1 h infusion of PD 156707 a challenge bolus dose of 0.3 nmol/kg of human endothelin-1 was administered intravenously. Fig. 1 is a representative example of one control experiment. In the control group, the endothelin-1 challenge produced a significant decrease in mean arterial blood pressure within the first minute, which was followed by a gradual return to normal levels in mean arterial pressure 5 min after the challenge. The systemic hypotensive response was characterized by a maximum decrease of 19 ± 3 mmHg in mean arterial blood pressure. The most dramatic change was the rapid increase in renal vascular resistance (increasing an average of 6.4 ± 1.0 mmHg/ml per min), consistent with the profound vasoconstrictor effects of endothelin-1 on the renal vasculature. Renal blood flow decreased precipitously to approximately 20% of the baseline value, then gradually returned to normal levels (Fig. 1).

In all of the PD 156707 treated animals, the early endothelin-1 induced responses were similar to those of the controls with regards to mean arterial blood pressure.

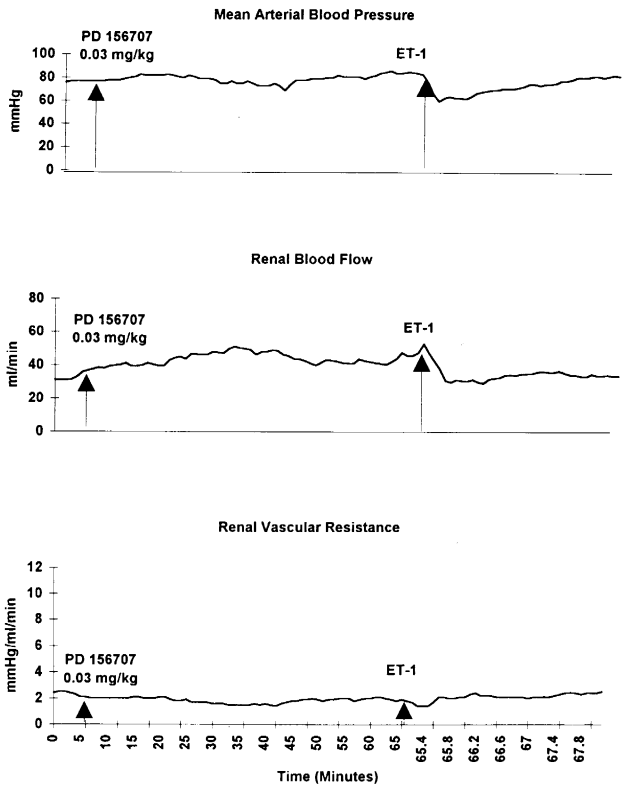

Fig. 2. A typical example of mean arterial blood pressure, renal blood flow, and renal vascular resistance over time in a PD 156707 (0.03 mg/kg per h) experiment. The arrows indicate when PD 156707 and endothelin-1 (ET-1) were given. Note that the endothelin-1 induced reductions in mean arterial blood pressure and renal blood flow are similar to the control experiment (Fig. 1). Most notably, however, there is no increase in renal vascular resistance with endothelin-1 in the presence of 0.03 mg/kg per h PD 156707.

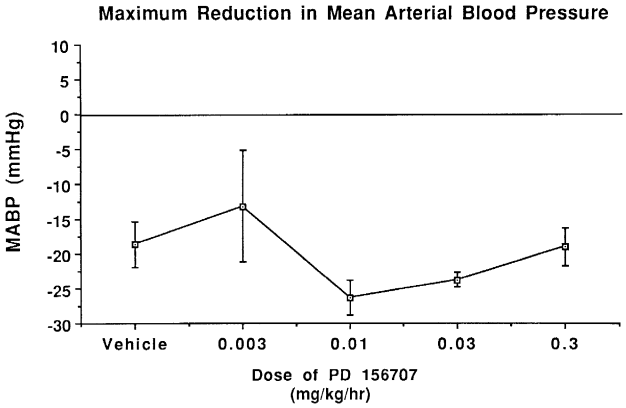

Fig. 3. Maximum reduction in mean arterial blood pressure (mmHg) occurring after the endothelin-1 bolus administration in all groups. The line graph illustrates the vasodilatory ('endothelin ET_B receptor') response for each dose as an absolute change from Post-Rx. This reponse is the maximal negative change in mean arterial blood pressure that is normally seen within seconds after endothelin-1 infusion. This response was similar among all groups.

The major effect of PD 156707 was blockade of the increase in renal vascular resistance evoked by the endothelin-1 challenge (Table 2). Fig. 2 is a representative example of one PD 156707 experiment in which the dose was 0.03 mg/kg per h. The reduction in mean arterial blood pressure evoked by endothelin-1 is evident and is followed by a gradual restoration to baseline levels. Renal blood flow decreased, largely following the change in mean arterial blood pressure. Most notably, there was virtually no change in renal vascular resistance, consistent with the interpretation that complete blockade of endothelin ET_A receptors in the renal vascular bed had been achieved.

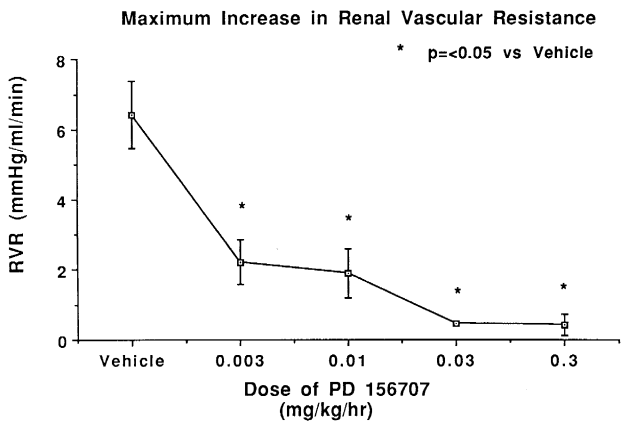

Fig. 4. Maximum increase in renal vascular resistance occurring after the endothelin-1 bolus administration in all groups. The line graph illustrates the vasoconstrictor ('endothelin ET_A receptor') response for each dose as an absolute change (in mmHg/ml per min) from Post-Rx. This reponse is the maximal increase in mean renal vascular resistance that is normally seen within 3 min after the endothelin-1 infusion. A marked increase in renal vascular resistance was significantly attenuated at 0.003 and 0.01 mg/kg per h, and almost completely eliminated at 0.03 and 0.3 mg/kg per h dose levels of PD 156707.

Fig. 3 summarizes the average changes in mean arterial blood pressure following the bolus administration of endothelin-1, in all of the groups. The graph presents the absolute reduction in mean arterial blood pressure that was normally observed within seconds after the endothelin-1 bolus. The depressor response associated with the endothelin-1 challenge was similar in all groups, including the vehicle group, supporting the interpretation that endothelin ET_B receptors were not blocked by PD 156707 at the doses used in this study. Fig. 4 summarizes the absolute changes in renal vascular resistance following the endothelin-1 bolus challenge, in all of the groups. The graph presents the vasoconstrictive response for each dose as an absolute change from Post-Rx values. There was complete elimination of the endothelin-1 induced increase in renal vascular resistance at doses of 0.03 and 0.3 mg/kg per h, and partial but significant inhibition at doses of 0.003 and 0.01 mg/kg per h.

4. Discussion

The major objective of this study was to determine the minimum effective dose of PD 156707, a nonpeptide endothelin ET_A receptor antagonist, for blocking the effects of exogenously administered endothelin-1 in the rabbit. When endothelin-1 was administered intravenously as a bolus, it elicited a reduction in mean arterial blood pressure ('endothelin ET_B receptor response') followed by a gradual return to baseline levels. Although a systemic hypotensive response was elicited by the endothelin-1 bolus, there was a dramatic increase in renal vascular resistance in control animals. The response to exogenous administration of endothelin-1 in rats (Yanagisawa et al., 1988; Inoue et al., 1989) is usually biphasic, characterized by a very transient decrease in mean arterial blood pressure followed by a sustained increase in mean arterial blood pressure. The delayed increase in mean arterial blood pressure was not apparent in the present study conducted with rabbits, but the depressor effect was consistently observed, suggesting that there are species differences in responses to exogenous endothelin-1. Pretreatment with PD 156707 did not attenuate the reduction elicited by endothelin-1 in mean arterial blood pressure, supporting our interpretation that endothelin ET_B receptors were not blocked at the doses used in this study. The renal vasoconstrictor response produced by exogenous endothelin-1 was blocked, however, indicating that the effects of the compound were exerted primarily on endothelin ET_A receptors. We observed endothelin ET_A receptor blockade, defined by effects on renal vascular resistance, at all four doses of PD 156707, including the two lower doses at which plasma concentrations could not be detected. Nearly complete blockade was evident at 0.03 mg/kg per h (corresponding to a plasma concentration of approximately 25 ng/ml). Less complete but significant blockade was produced by 0.01 and 0.003 mg/kg per h leading us to conclude that the minimum effective dose of PD 156707, to achieve complete endothelin ET_A receptor blockade, is in the range of 0.01 and 0.03 mg/kg per h.

Another objective of this study was to evaluate the utility of renal vascular resistance in the rabbit as a bioassay of endothelin ET_A selective antagonism. Endothelin-1 causes increases in vascular resistance in the vascular beds of the testes, epididymis, skeletal muscle, skin, spleen, kidneys, intestines and pancreas/mesentery. When the proportion of the cardiac output received by the individual beds is taken into account, the renal vascular bed is a major contributor to the pressor effects of endothelin-1 observed in the rat, when administered at high enough doses (Walder et al., 1989). In this species, the kidney appears to be approximately 10-times more sensitive than other organs to the vasoconstrictor effects of endothelin-1 (Pernow et al., 1989; Kon et al., 1989). Dose dependent increases in mean arterial blood pressure, and elevated renal, coronary, femoral and bronchial vascular resistances were observed with intravenously administered endothelin-1 (2–400 pmol/kg) in the pig, as well, but the vascular bed of the kidney remained the most responsive to endothelin-1 (Pernow et al., 1989). In addition, the potency of endothelin-1 as a vasoconstrictor in the renal bed of the rabbit has been reported by Rae et al. (1989) consistent with the results of the present study. These findings, obtained in three different species, demonstrate that changes in renal vascular resistance represent a useful means of assaying the functional effects of endothelin ET_A selective antagonists such as PD 156707.

The endothelin ET_A antagonistic effects of PD156707 have largely been studied in vivo using rats. Interpretation of endothelin ET_A selective effects of this compound in the rat, however, is complicated by two issues. First, based on binding studies, the endothelin ET_A selectivity of PD 156707 in the rat is relatively poor. We minimized this problem in the present study by using rabbits, a species in which PD 156707 is 1300-fold selective for endothelin ET_A receptors versus endothelin ET_B receptors (Reynolds et al., 1995a). Second, in the rat the effects of endothelin receptor antagonism are often examined by measuring the arterial blood pressure changes elicited by exogenous endothelin-1. This poses a potential problem because mean arterial blood pressure integrates into one parameter a variety of effects that may differ greatly from organ to organ, because the blood pressure response to exogenous endothelin-1 represents a mixture of endothelin ET_A and ET_B mediated changes (Bigaud and Pelton, 1992; Cornet et al., 1993; Reynolds et al., 1995a; Doherty et al., 1995), and because a significant arterial pressor response is not evident in all species, as we noted using rabbits in the present study. To minimize these problems, we focused on the renal vascular bed in which endothelin mediated control of blood flow is primarily mediated by endothelin ET_A receptors, simplifying interpretation of the results. Another

advantage of using renal vascular resistance is that maximum blockade corresponded to nearly 100% abolition of the increase in vascular resistance produced by the exogenous endothelin-1 challenge. Thus, the potential 'signal' that can be detected using a bioassay based on a renal vascular resistance is large, extending over a wide range (0–100% blockade). The range of the 'signal' using blockade of the endothelin-1 induced arterial pressor response in rats is somewhat smaller, being restricted to reductions of 50–60% by endothelin ET_A selective compounds such as PD 156707 or BQ-123 (Reynolds et al., 1995a).

In conclusion, the results of this study demonstrate that PD 156707 is an extremely potent and highly selective endothelin ET_A receptor antagonist. A dose of 0.03 mg/kg per h completely abolished increases in renal vascular resistance induced by an intravenous challenge of 0.3 nmol/kg of human endothelin-1, without attenuating the peak arterial pressure reduction characteristic of the endothelin ET_B receptor. At doses lower than 0.03 mg/kg per h, PD 156707 was not detected in the plasma but did produce partial blockade of the increase in renal vascular resistance due to endothelin-1. In addition, this study demonstrates that renal vascular resistance is a sensitive marker for evaluating the effects of endothelin ET_A receptor antagonism in this in-vivo rabbit model.

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