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In vivo pharmacology of an angiotensin AT_1 receptor antagonist with balanced affinity for angiotensin AT_2 receptors

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Abstract

L-163,017 (6-[benzoylamino]-7-methyl-2-propyl-3-[[2′-(*N*-(3-methyl-1-butoxy)carbonylaminosulfonyl)[1,1′]-biphenyl-4-yl]-methyl]-3*H*-imidazo[4,5-*b*]pyridine) is a potent, orally active, nonpeptide angiotensin II receptor antagonist. Conscious rats and dogs were dosed p.o. and i.v.; in both species the plasma bioequivalents are similar at the angiotensin AT_1 and AT_2 receptor sites indicating balanced activity is maintained in vivo. L-163,017 prevents the pressor response to intravenous (i.v.) angiotensin II in the conscious rat, dog, and rhesus monkey. L-163,017 also significantly reduces blood pressure in a renin-dependent model of hypertension, similar to an angiotensin converting enzyme inhibitor (Enalapril) and an angiotensin AT_1 receptor-selective antagonist (L-159,282). These studies indicate that neither the angiotensin AT_2 receptor nor bradykinin is important in the acute antihypertensive activity of angiotensin converting enzyme inhibitors or angiotensin II receptor antagonists.

Keywords: Angiotensin II; Angiotensin receptor antagonist; Angiotensin converting enzyme inhibitor; Antihypertensive; L-163,017

1. Introduction

The potential for multiple angiotensin II receptor subtypes has been recognized for some time. Evidence for two distinct subtypes of angiotensin II receptors originated from the differential affinities for antagonists such as losartan and L-158,809 compared to PD-123177 and analogs (Chang and Lotti, 1990; Chiu et al., 1989; Whitebread et al., 1989). The angiotensin II receptor subtype with high affinity for losartan has been designated the angiotensin AT_1 receptor and the binding site having high affinity for PD-123177 as the angiotensin AT_2 receptor (Bumpus et al., 1991). Recently, both the angiotensin AT_1 and AT_2 receptors have been cloned and shown to possess approximately 35% sequence homology (Iwai et al., 1991; Murphy et al., 1991; Mukoyama et al., 1993; Kambayashi et al., 1993). It should be noted that other subtypes have been suggested as well as subtype heterogeneity (for review see Timmermans et al., 1993).

Although no clear function for the angiotensin AT_2 receptor binding site has been identified, recent reports in the literature provide insight into possible functional roles for this site (Kambayashi et al., 1994; Kang et al., 1994; Brilla et al., 1994). Binding experiments have now established that the number of angiotensin AT_2 receptor sites is greater in the developing embryo and neonate compared to the adult (Tsutsumi and Saavedra, 1991; Schambelan et al., 1991). These data suggest that the angiotensin AT_2 receptor site may play a role in growth and development. In keeping with this theory, recent investigations have found that the angiotensin AT_2 receptor binding site is upregulated following injury. The density of the angiotensin AT_2 receptor binding site has been shown to increase following balloon angioplasty and a role for the angiotensin AT_2 receptor site in proliferation of

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the neointima has been suggested (Janiak et al., 1992). Arterial hypertension and heart failure, activated states of the renin-angiotensin system are associated with myocardial fibrosis. However, the importance of angiotensin II as a hormonal stimulus of cardiac growth and collagen synthesis remains unclear. Angiotensin II-stimulated collagen synthesis has been suggested to be mediated via the angiotensin AT_2 receptor binding site (Brilla, 1992) indicating a physiologically important role for the angiotensin AT_2 receptor site in altering cardiac fibroblast function to promote myocardial fibrosis during states of increased renin-angiotensin system activity. It should also be noted that others have not demonstrated an angiotensin AT_2 receptor antagonist-mediated effect of angiotensin II in cardiac fibroblasts (Schorb et al., 1992; Matsubara et al., 1994; Crabos et al., 1994). Acute sodium depletion also increases the activity of the renin-angiotensin system. In sodium-depleted dogs, PD123319 (angiotensin AT_2 receptor selective) has been shown to increase free-water clearance in a dose-dependent manner (Keiser et al., 1992). PD123177, a chemically related analog of PD123319 which is also angiotensin AT_2 receptor selective, has been reported to inhibit the reabsorption of chloride and water in the early S_1 segment of the proximal tubule (Cogan et al., 1991). Promising experimental results, such as these, have led to the wide acceptance of the angiotensin AT_2 receptor binding site as an angiotensin II receptor subtype. Therefore, we shall refer to the angiotensin AT_2 site as a receptor, although no in vivo biological function has been identified. The studies mentioned above raise important questions about the role of the angiotensin AT_2 receptor in normal physiology and in the pathophysiology of disease.

Studies designed to elucidate a physiological function for the angiotensin AT_2 receptor have used angiotensin AT_1 and AT_2 receptor antagonists in combination. An inherent problem in this approach is the inability to demonstrate functional blockade of the angiotensin AT_2 receptor antagonist. As a result these types of studies require dosing based upon plasma drug levels. For this approach to work, there must be a correlation between the plasma drug level, receptor occupancy and blockade. It was our goal to discover a compound that is equipotent at both the angiotensin AT_1 and AT_2 receptors and that retains this balanced profile following in vivo dosing. Such a compound would then allow one to dose according to the inhibition of the pressor response to exogenously administered angiotensin II (angiotensin AT_1 receptor mediated) with the knowledge that, when this response is blocked, we would also have occupancy at the angiotensin AT_2 receptor.

Additionally, an important issue is whether a balanced (i.e. equipotent at both angiotensin AT_1 and AT_2 receptors) angiotensin receptor antagonist will have advantages over either an angiotensin converting enzyme inhibitor or an angiotensin AT_1 receptor-selective antagonist for the treatment of hypertension, heart failure and other disease states. Angiotensin converting enzyme inhibitors are functionally balanced since, by preventing the formation of angiotensin II, they block the action of angiotensin II at all receptor sites. However, recent evidence suggests that angiotensin II can be formed via angiotensin converting enzyme-independent pathways (Urata et al., 1990). A balanced angiotensin II receptor antagonist would block the effects of angiotensin II without regard to its synthetic pathway. In addition, a balanced angiotensin II receptor antagonist should be devoid of bradykinin potentiation and, therefore, be more specific in its action. It is also expected that such a compound will not produce angioedema or the dry cough often associated with angiotensin converting enzyme inhibitor therapy in man.

Therefore, the angiotensin AT_2 receptor may play an important role in both normal physiology and the pathophysiology of disease. For the reasons sited above, a compound with balanced affinity for both the angiotensin AT_1 and AT_2 receptors should offer an advantage over currently available pharmacologic tools and provide insight as to the role of the angiotensin AT_2 receptor. In the present study we describe the in vivo pharmacology of a newly discovered angiotensin II receptor antagonist, L-163,017, (6-[benzoylamino]-7-methyl-2-propyl-3-[[2′-(*N*-(3-methyl-1-butoxy)carbonylaminosulfonyl) [1,1′]-biphenyl-4-yl]methyl]-3*H*-imidazo-[4,5-*b*]pyridine) (Fig. 1) with balanced affinity for both the angiotensin AT_1 and AT_2 receptors (Chang et al., 1995). Because the in vitro potency of L-163,017 for both receptor subtypes is approximately equal and remains balanced following in vivo administration, the

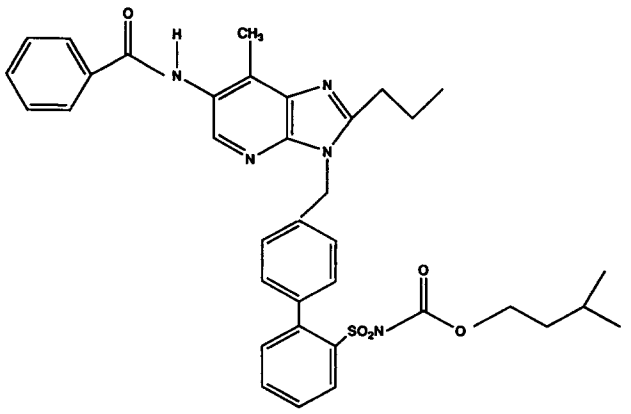

Fig. 1. The chemical structure of L-163,017 (6-[benzoylamino]-7-methyl-2-propyl-3-[[2′-(*N*-(3-methyl-1-butoxy)carbonylaminosulfonyl)-[1,1′]-biphenyl-4-yl]methyl]-3*H*-imidazo[4,5-*b*]pyridine).

aim of the present experiments was to gain insight into the role of the angiotensin AT_2 receptor in mediating the biological response to angiotensin II and the role of bradykinin in the antihypertensive activity of angiotensin converting enzyme inhibitors.

2. Materials and methods

2.1. In vivo receptor occupancy

In vivo competition studies were conducted as described in previous publications from our laboratories, with minor modifications (Gibson et al., 1994). Male Sprague-Dawley rats (200–250 g) were anesthetized with ketamine/acepromazine and pretreated with Enalapril (10 mg/kg i.p.) to reduce endogenous levels of angiotensin II. Rats were treated with a single intravenous dose of test compound or vehicle. Ten minutes after dosing, 5 μCi of ^{125}I[Sar^1,Ile^8]angiotensin II was administered intravenously and 1 h later, rats were killed. Plasma samples, one kidney and both adrenals were collected on ice. Tissues were trimmed of fat and counted in an autogamma counter. The data were converted to percent-injected dose/g wet weight of tissue and expressed as the percent of receptor-specific signal (total radioactivity present in tissue radioactivity in the presence of saturating antagonist). Dose vs. receptor occupancy curves were generated using a minimum of four doses each of test compound. Potency of receptor occupancy was expressed as an IC_{50} value (dose of test compound where 50% of specific ^{125}I[Sar^1,Ile^8]angiotensin II binding is displaced).

2.2. Maintenance of balanced activity in vivo and oral bioavailability

Fasted female mongrel dogs (8–10 kg) were implanted with vascular access ports prior to the study. The vascular access port allowed direct monitoring of blood pressure and heart rate. On the day of the experiment, percutaneous catheters were inserted, using sterile technique, in the saphenous veins for the intravenous administration of L-163,017 and test challenges with either angiotensin II or norepinephrine. Following calibration of the pressure transducers and an appropriate period of equilibration, dogs were challenged with bolus doses of angiotensin II (0.1 μg/kg) and norepinephrine (1.6 μg/kg) to ensure patency of the catheters and responsiveness of the preparation. Dogs were then dosed orally or intravenously at 5.0 mg/kg with L-163,017. Functional blockade of the angiotensin AT_1 receptor was demonstrated by inhibition of the pressor response to exogenous angiotensin II and selectivity of L-163,017 by the lack of inhibition of the pressor response to norepinepherine. Blood was collected (2, 5, 15, 30 min and 1, 2, 4, 8, and 24 h after dosing) in EDTA at 4°C for measurement of drug level via radioreceptor assay and calculation of plasma half-life and oral bioavailability.

Plasma was separated by centrifugation. To the plasma samples (0.5 ml each), 2.5 ml of methanol was added, mixed vigorously, and then centrifuged at 3000 $\times g$ for 15 min. Aliquots (2.0 ml) of the supernatants were carefully removed and dried with a Speedvac. The dried residues were dissolved in 0.5 ml dimethyl sulfoxide (DMSO); if further dilution was necessary the same solvent was used. Aliquots (10 μl) were used in a competitive binding assay with ^{125}I[Sar^1,Ile^8]angiotensin II at both the angiotensin AT_1 and AT_2 receptors in rat adrenal membranes, as previously described (Chang and Lotti, 1990, 1991; Chang et al., 1995). L-163,017 equivalents in the plasma samples were estimated by comparing the percent inhibition of binding produced by the samples with known standard curves (0.01–1 mg/ml) of L-163,017.

2.3. Conscious normotensive rats

Male Sprague-Dawley rats (300–400 g) were anesthetized with a short-acting barbiturate (methohexital; 50 mg/kg i.p.) and instrumented with chronic vascular catheters 24 h prior to the experiment. A catheter placed in the abdominal aorta, via the femoral artery, was used for the direct measurement of blood pressure and pulse rate. Two catheters placed in the inferior vena cava, via the femoral vein, were used for the intravenous (i.v.) administration of L-163,017 and challenges with angiotensin II or methoxamine. A subcutaneous tunnel was created and all catheters were exteriorized between the scapulae and protected with a Harvard tethering system. This system allows the animals movement within the cage. Rats were permitted to recover overnight from anesthesia and allowed free access to water. Food was withheld when L-163,017 was administered orally.

Following calibration of the pressure transducers and an appropriate equilibration period, bolus i.v. doses of angiotensin II (0.1 μg/kg, submaximal challenges) were administered to determine control responses and patency of catheters. Each rat was then treated with a single p.o. or i.v. dose of L-163,017 and any changes in blood pressure were noted. The percentage of inhibition of the pressor response to angiotensin II challenges during the subsequent 6 h and at 24 h was used as a measure of angiotensin II inhibition. Inhibition of the pressor response to methoxamine was used to assess selectivity of the L-163,017 for angiotensin II receptors. Relative potency was quantitated using ED_{50} values (effective dose to inhibit 50% of the control response), which were calculated from the peak inhibition observed at several doses of the test compound.

2.4. Conscious sodium / volume-depleted rhesus monkeys

Male and female rhesus monkeys (2.7–7 kg) were surgically instrumented with chronic arterial catheters with access ports (Access Technologies), at least 2 weeks before the experiment, as previously described (Siegl et al., 1992). The monkeys were maintained on a low-sodium diet (< 2 mEq/day) for one week and injected with Lasix (5 mg/kg i.m.) 18 h prior to the experiment. Food was denied at this time; water was allowed ad libitum.

On the morning of the experiment, the monkeys were placed in chairs with minimal restraint. Aseptic procedures were employed during the entire experiment. Alcohol, betadine, and alcide were used to wash the monkey's skin surrounding and covering the access ports. Sterile Huber point needles (20 gauge, without hubs), attached to tubing were inserted into the ports. The arterial tubing was attached to a Statham blood pressure transducer (Spectramed) for continuous monitoring of mean arterial pressure and heart rate using Buxco-IBM software and recorder. Two sterile catheters were inserted in the saphenous or brachial vein for administration of angiotensin II and L-163,017. All solutions injected into the animals were obtained sterile or filtered using an Acrodisc filter (0.2 μm).

The monkeys were initially challenged with methoxamine (50 μg/kg i.v.) to determine a basal response to an agonist other than angiotensin II. Bolus injections of angiotensin II (0.1 μg/kg i.v.) were given at −45, −30, and −15 min to determine the control response for angiotensin II in these animals. L-163,017 or its vehicle was then administered. Angiotensin II challenges were given (i.v.) at 5, 15, and 30 min and every half-hour thereafter for 6 h. When the duration of activity necessitated continuing the study overnight, the angiotensin II catheter was flushed with 5% dextrose/H_2O (D5W) to remove the angiotensin II, and then methoxamine (50 μg/kg i.v.) was administered to verify patency of the angiotensin II catheter and confirm specificity of the antagonist. The next morning, methoxamine was administered, to verify patency of the catheter. The catheter was then reloaded with the same solution of angiotensin II (stored overnight at 4°C). The monkey was challenged 3 times with angiotensin II, 15 min apart; the response to the last challenge was used for the 24 h time point.

2.5. Effect on bradykinin-induced blood pressure response

The effect of L-163,017 on blood pressure responses to bradykinin was compared to that of enalaprilat in conscious rats. Male Sprague Dawley rats (200–400 g) from Taconic Farms were anesthetized with brevital (50 mg/kg i.p.). Catheters were placed in the left femoral artery and right common carotid artery for the measurement of arterial pressure and the administration of bradykinin, respectively. A catheter was placed in the left femoral vein for the administration of nitroglycerin. The right femoral vein was catheterized for the administration of either vehicle (normal saline), enalaprilat or L-163,017, and for the administration of angiotensin I. Catheters were tunneled subcutaneously and exteriorized between the scapulae. The catheters were protected by a spring tether. Rats were allowed to recover overnight. Food and water were available ad libitum throughout the night and water was available ad libitum during the experiment.

Mean arterial pressure in the conscious rat was measured via the femoral artery catheter using a Statham (P23Gb) pressure transducer coupled to a Hewlett-Packard recording system. The pressor response to angiotensin I (0.2 μg/kg i.v.) was tested. After a return to baseline pressure, dose-response curves to nitroglycerin (10, 30, 100 μg/kg i.v.) and bradykinin (1, 3, 10 μg/kg i.a.) were determined. Enalaprilat (3 mg/kg i.v.), L-163,017 (3 mg/kg i.v.), or vehicle was administered and 30 min later the nitroglycerin and bradykinin doses were repeated. Prior to and following the nitroglycerin and bradykinin doses, the pressor response to the angiotensin I challenge was determined to verify efficacy of the antagonists.

All drugs were made fresh the day of the experiment. Enalaprilat and angiotensin I were dissolved in 0.9% saline and administered in a volume of 1.0 ml/kg. L-163,017 was dissolved in 0.45% saline and administered in a 2 ml/kg volume. The doses of nitroglycerin and bradykinin were administered by giving increasing volumes (0.2 ml/kg (low dose), 0.69 ml/kg (middle dose), or 2.0 ml/kg (high dose)) of stock solutions (50 μg/ml and 15 μg/ml for nitroglycerin and bradykinin, respectively).

2.6. Renin-dependent hypertensive rats

Male Sprague-Dawley rats (160–250g) were anesthetized with sodium pentobarbital (35 mg/kg i.p.). When a surgical plane of anesthesia was reached, the ventral abdominal region was shaved and cleaned with 70% alcohol and betadine. Under aseptic conditions, a ventral midline incision was made, and the contents of the abdomen moved until the descending aorta was visualized. The descending aorta was isolated in the area between the left and right renal bifurcation. A 4-0 suture was passed under the aorta and tied securely to completely occlude the aorta. The left kidney became pale and the right kidney remained pink indicating a successful ligation. The left kidney was not removed during this procedure. Following ligation, the intestines were placed back in the abdomen, the muscle layer sutured with 2-0 silk, the skin closed with staples, and

the wound site cleaned with betadine. The rat was checked each day for the next 6 days. If the hindquarters on any of the rats showed paralysis, the rat was humanely killed and not used in the study.

Following 1 week of coarctation, the surviving rats were anesthetized with brevital (35 mg/kg i.p.). A ventral neck incision was made and PE-50 catheters were placed in the right carotid artery and right external jugular vein for measurement of blood pressure, heart rate and compound administration, respectively. The catheters were filled with heparinized saline (10 units/ml), plugged, tunneled to the nape of the neck, externalized and passed through an Alice-King Chatham spring protector. The wound site was closed and the spring guard anchored with 2-0 suture. Upon recovery, the rats were placed in individual Plexiglas cages specifically designed for conscious blood pressure and heart rate monitoring.

The next morning the arterial catheter was connected to a Cobe Pressure Transducer and Grass Physiological recorder for the continuous monitoring of blood pressure and heart rate. Following the control

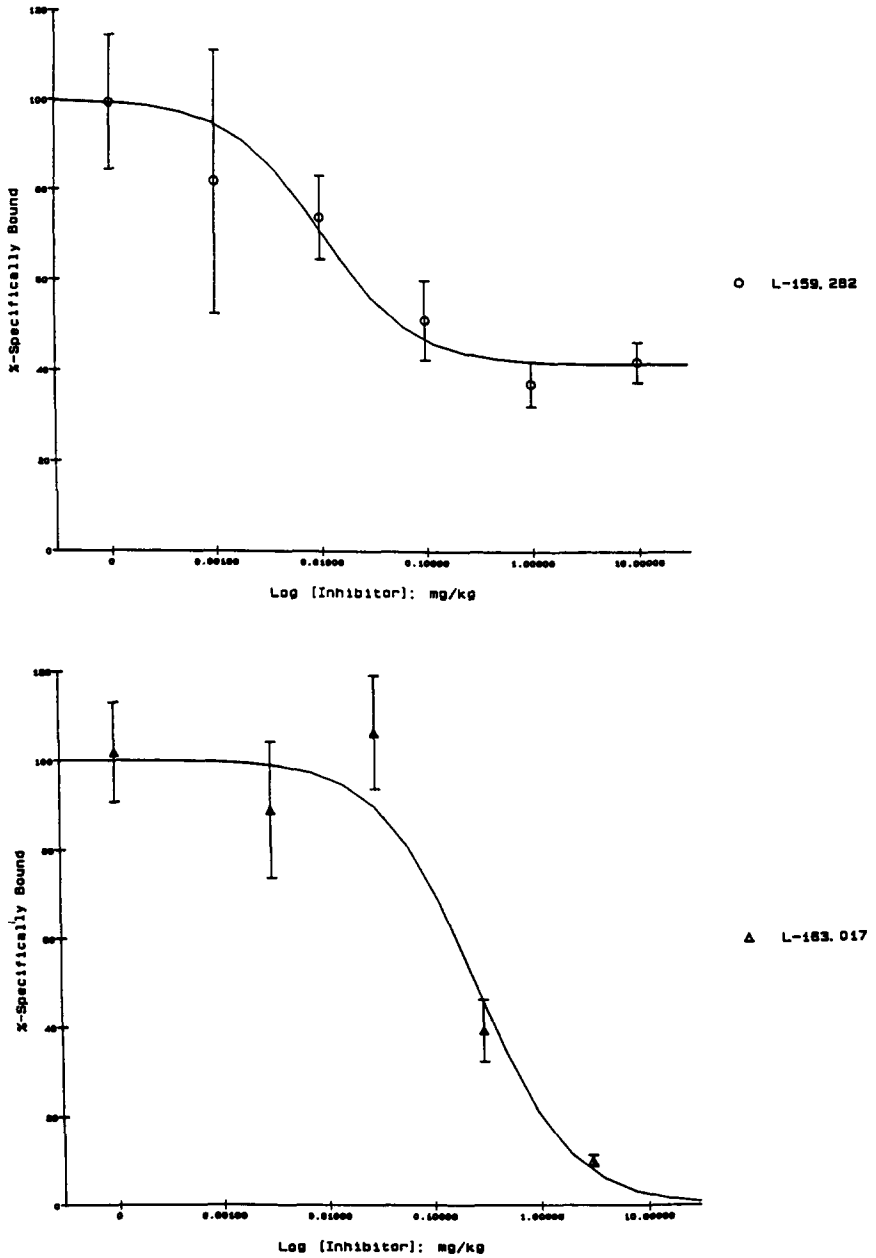

Fig. 2. The in vivo blockade of ^{125}[Sar^{8},II e]angiotensin II binding to rat adrenals by L-159,282 (top panel) and L-163,017 (bottom panel). L-163,017 displaces 100% of the specific binding whereas L-159,282 (angiotensin AT_1 receptor selective) displaces approximately 50% of the specific binding in this tissue known to contain both angiotensin AT_1 and AT_2 receptors. Dose vs. receptor occupancy curves were generated using a minimum of four doses, in four separate animals, of each test compound.

period, the rat was given an oral dose (3.0 mg/kg, gavage) of L-163,017, enalapril, MK-996 (angiotensin AT_1 receptor selective) (Kivlighn et al., 1995) or vehicle (H_2O, 10 ml/kg). Blood pressure and heart rate were continuously monitored for 6 h following treatment. After 6 h, the catheters were plugged. The next day, the arterial catheter was reconnected to the transducer and polygraph and a 24 h blood pressure measurement recorded.

2.7. Statistical analysis

Data are expressed as the mean ± standard error of the mean (S.E.M.). Statistical analyses used were analysis of variance with Scheffe's F-test for multiple comparisons, to assess significance between groups, and analysis of variance for repeated measures with Dunnett's t-test, to assess significance within groups compared to baseline. Differences were considered significant at $P \leq 0.05$.

3. Results

3.1. In vivo receptor occupancy

In vivo receptor occupancy of L-163,017 was studied in the rat. Adrenal tissue was used as a reporter tissue since it contains approximately equal numbers of angiotensin AT_1 and AT_2 receptors (Chang and Lotti, 1990, 1991). L-163,017 completely inhibited receptor-specific ^{125}I[Sar^1,Ile^8]angiotensin II binding with a monophasic dose-receptor occupancy curve (Fig. 2, lower panel). These results are good evidence that angiotensin II binding to both angiotensin AT_1 and AT_2 receptors is blocked in vivo by L-163,017. In comparison, the angiotensin AT_1 receptor-selective antagonist L-159,282 (MK-996) (Chang et al., 1994) blocked only 50% of ^{125}I[Sar^1,Ile^8]angiotensin II in rat adrenal (Fig. 2, upper panel). When angiotensin AT_1 receptors in the adrenal are blocked via pretreatment with 1 mg/kg (i.v.) L-159,282, the potency of L-163,017 (IC_{50} = 0.26 mg/kg) is identical to its potency when angiotensin AT_1 receptors are not blocked. Therefore, L-163,017 is equipotent in the displacement of ^{125}I[Sar^1,Ile^8]angiotensin II from both angiotensin AT_1 and AT_2 receptors in vivo.

3.2. Maintenance of balanced activity in vivo and oral bioavailability

An assay was developed to demonstrate that L-163,017 remains equipotent at both the angiotensin AT_1 and AT_2 receptors (i.e. balanced) throughout its functional duration of action. The method is based upon the principle that L-163,017 or its equivalent is present in the plasma of an animal following dosing and after extraction from the plasma, L-163,017 or its equivalent, should displace angiotensin II from both angiotensin AT_1 and AT_2 receptors in a radioreceptor assay. The assay is quantified by comparison to standard curves with known amounts of L-163,017, in vitro. Angiotensin AT_1 and AT_2 receptor antagonist plasma equivalents following intravenous administration of L-163,017 (5.0 mg/kg) are shown in Fig. 3. The plasma bioequivalents are the same at both the angiotensin AT_1 and AT_2 receptors demonstrating that balanced angiotensin AT_1/AT_2 receptor antagonist activity is maintained in vivo in the dog. Identical results were obtained using this experimental protocol in the rat (data not shown). In addition, this assay allowed us to calculate the bioavailability of L-163,017 in both the rat and dog. From these data the oral bioavailability of L-163,017 is estimated to be 45% in the rat and 23% in the dog.

3.3. In vivo potency and duration

In conscious normotensive rats, oral or intravenous administration of L-163,017 (0.1–5.0 mg/kg) significantly inhibits angiotensin II-induced pressor responses (Fig. 4). In these animals L-163,017 did not change basal blood pressure or affect the pressor response to methoxamine (an α-adrenoceptor agonist). In the conscious rat, L-163,017 has an oral (p.o.) ED_{50} = 0.29 mg/kg and an intravenous (i.v.) ED_{50} = 0.28 mg/kg (Table 1). Losartan has a p.o. ED_{50} = 0.66 and an i.v. ED_{50} = 0.28 and MK-996 has p.o. and i.v. ED_{50} values = 0.067 and 0.014 mg/kg respectively (Table 1). Therefore, L-163,017 has a p.o./i.v. ratio = 1.0, compared to losartan p.o./i.v. ratio = 2.4 and MK-996 p.o./i.v. ratio = 4.8. These data suggest that L-163,017

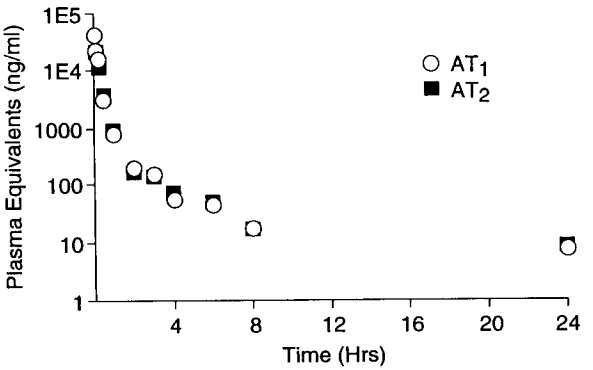

Fig. 3. Angiotensin AT_1 and AT_2 receptor antagonist plasma equivalents following intravenous administration of L-163,017 (5 mg/kg) in dogs. The plasma bioequivalents are similar at both angiotensin AT_1 and AT_2 receptors for the 24 h period after dosing indicating that L-163,017 remains balanced following administration in dogs. These results are the mean of five experiments conducted in different animals.

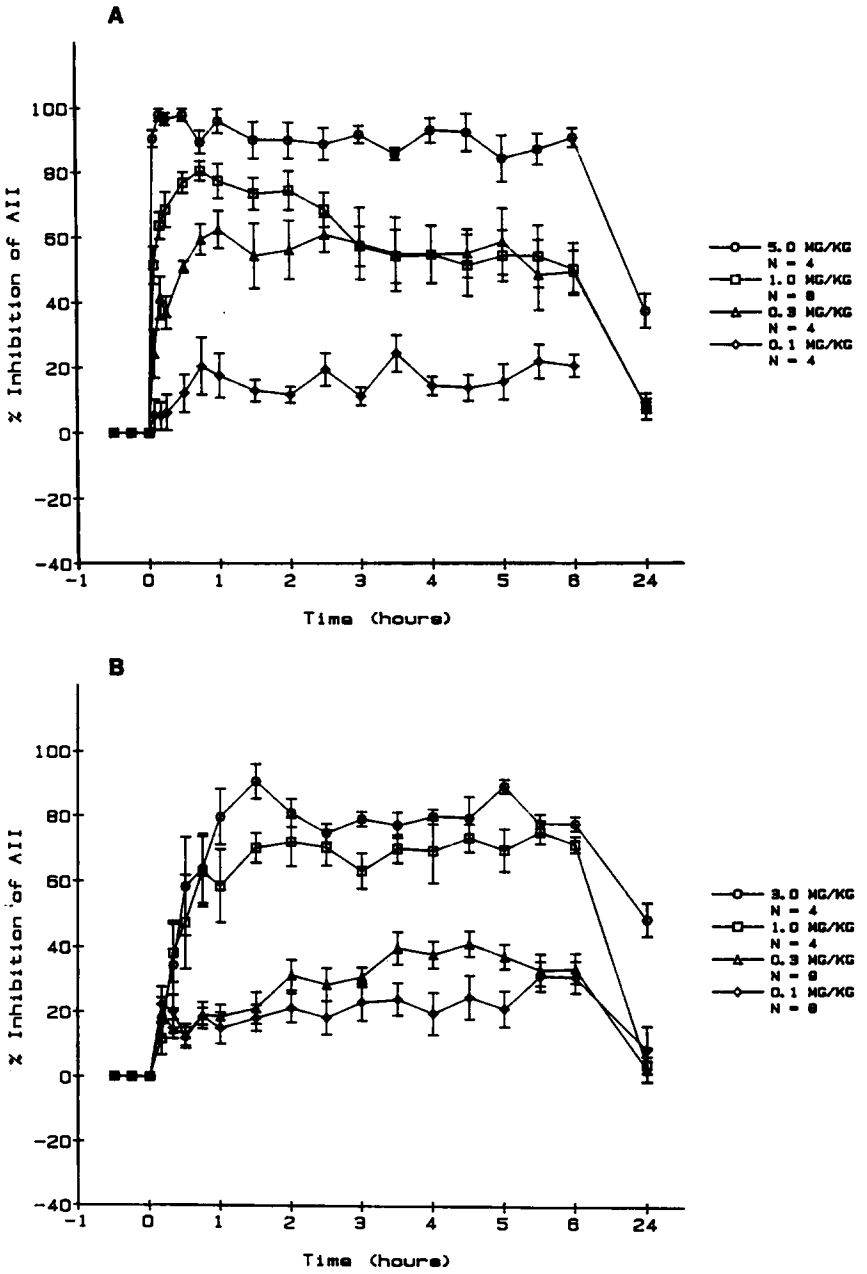

Fig. 4. Inhibition of angiotensin II-induced pressor responses following intravenous (panel A) and oral (panel B) administration of L-163,017 to conscious normotensive rats. Each rat received a single dose of L-163,017. Data are expressed as mean ± S.E.M.

Table 1
Relative in vitro and in vivo potencies and oral bioavailability of MK-954 (losartan), MK-996 (L-159,282), and L-163,017

	In vitro [a]			In vivo (rat)			
	IC_{50}			ED_{50} (mg/kg)			Oral bioavailability (%) (rat)
	AT_1	AT_2	+HSA	p.o.	i.v.	p.o./i.v.	
MK-954	26 nM	> 30 μM	400 nM	0.66	0.28	2.4	33
(E3174) [b]	(0.84 nM)	> 30 μM	(210)				
MK-996 (L159,282)	0.36 nM	> 1 μM	25 nM	0.067	0.014	4.8	25
L-163,017	0.13 nM	0.17 nM	52 nM	0.29	0.28	1.0	45

[a] In vitro data from Chang et al. (1985). [b] E3174 is the active metabolite of losartan (MK-954). ED_{50} values were calculated from responses with at least three doses of each compound and at least four animals/dose. Inhibition of the pressor response to exogenous angiotensin II was used as a measure of efficacy.

is more bioavailable following p.o. administration than either losartan or MK-996. Following an intravenous dose of 0.3 mg/kg, L-163,017 has a duration of action that exceeds 5 h with a peak inhibition of 72% of the pressor response to an exogenous angiotensin II challenge (Fig. 4). An oral dose of L-163,017 (1.0 mg/kg) produces 86% (peak) inhibition of an exogenous angiotensin II challenge and duration of action that exceeds 6 h (Fig. 4).

3.4. Conscious sodium / volume-depleted rhesus monkeys

Following intravenous administration to conscious sodium/volume-depleted rhesus monkeys, L-163,017 (1.0 mg/kg) inhibited pressor responses to exogenously administered angiotensin II and reduced renin-angiotensin system. The duration of action of L-163,017 exceeded 6 h following a peak response of 96% (inhibition of the pressor response to exogenous angiotensin II; Fig. 5). L-163,017 significantly reduced renin-angiotensin system (−35 mm Hg, peak effect) in these animals. The reduction in renin-angiotensin system was correlated to the percent inhibition of the pressor response to angiotensin II, suggesting that the reduction in renin-angiotensin system is related to the blockade of angiotensin II receptors by L-163,017.

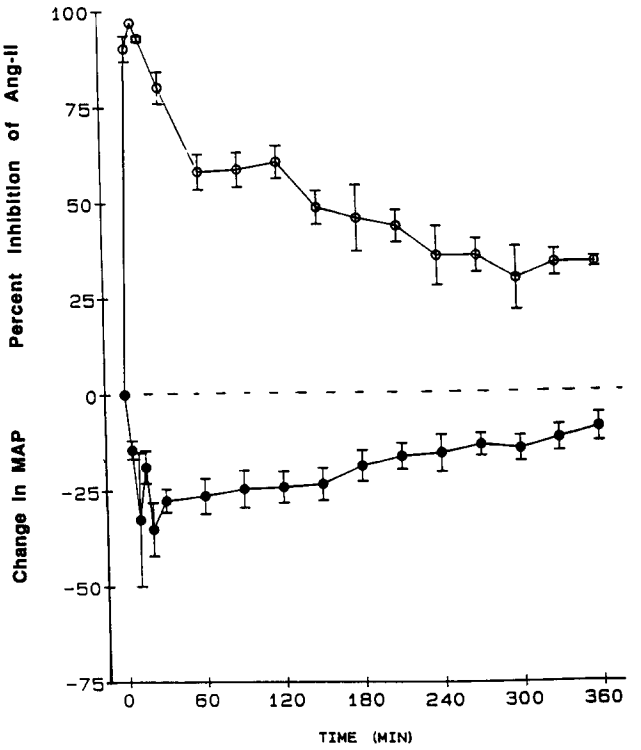

Fig. 5. Inhibition of angiotensin II-induced pressor responses and change in mean arterial pressure (renin-angiotensin system; mm Hg) in conscious sodium/volume-depleted rhesus monkeys following intravenous administration of L-163,017 (1 mg/kg). The data are presented as the mean ± S.E.M. ($n = 5$).

3.5. Antihypertensive activity in renin-dependent hypertension

Coarctation of the abdominal aorta produced hypertension in Sprague-Dawley rats 7 days after surgery. Pretreatment blood pressure in these animals was between 173 and 186 mm Hg (Fig. 6). Oral treatment with L-163,017 (3.0 mg/kg) significantly lowered blood pressure in these animals. Like L-163,017, both MK-996 (angiotensin AT_1-selective receptor antagonist) and enalapril (an angiotensin converting enzyme inhibitor) also significantly reduced renin-angiotensin system following oral dosing with 3.0 mg/kg (Fig. 6). The antihypertensive effect is of long duration following treatment with either L-163,017, MK-996 or enalapril. Blood pressure in the treated animals remained significantly below that of the vehicle-treated animals for at least 6 h after dosing (Fig. 6). Twenty-four hours after dosing, blood pressure in the treated animals remained 24–36 mm Hg below the vehicle-treated animals; however,

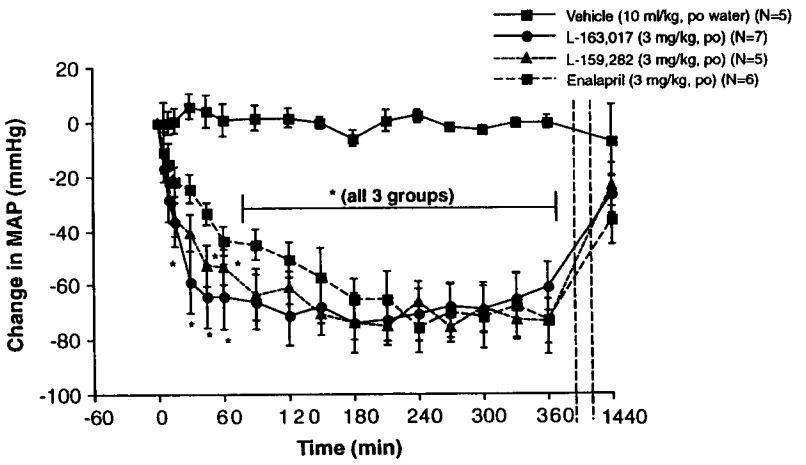

Fig. 6. Comparison of the antihypertensive effect of L-163,017, L-159,282 and enalapril in a renin-dependent model of hypertension, aortic coarctation in the rat. Data are expressed as mean ± S.E.M. $^*P \leq 0.05$.

Table 2
Change in mean arterial pressure in response to nitroglycerin and bradykinin before and after intravenous administration of vehicle, L-163,017 (3 mg/kg) or enalaprilat (3 mg/kg)

	Vehicle		L-163,017		Enalaprilat	
	Before	After	Before	After	Before	After
Nitroglycerin (μg/kg)						
10	−17 ± 1	−10 ± 1	−13 ± 3	−12 ± 2	−13 ± 2	−16 ± 3
30	−17 ± 2	−17 ± 2	−16 ± 1	−16 ± 2	−18 ± 2	−20 ± 3
100	−16 ± 1	−15 ± 2	−16 ± 2	−19 ± 2	−16 ± 2	−18 ± 3
Bradykinin (μg/kg)						
1	−10 ± 1	−11 ± 2	−9 ± 1	−10 ± 1	−14 ± 1	−37 ± 2 [a]
3	−14 ± 1	−18 ± 3	−15 ± 2	−18 ± 2	−17 ± 2	−41 ± 2 [a]
10	−20 ± 1	−23 ± 3	−18 ± 2	−23 ± 2	−22 ± 1	−45 ± 2 [a]

Values are mean ± S.E.M. Number of animals: vehicle ($n = 7$), L-163,017 ($n = 9$), enalaprilat ($n = 7$). [a] Significantly different from vehicle and L-163,017, $P < 0.05$.

these values were not found to be statistically different from the vehicle-treated animals.

3.6. Effect on bradykinin-induced blood pressure response

Changes in renin-angiotensin system following nitroglycerin and bradykinin challenges are shown in Table 2. Bolus administration of bradykinin and nitroglycerin elicited decreases in renin-angiotensin system. The hypotension produced by nitroglycerin, a kinin-independent vasodilator, was not significantly different following administration of vehicle, L-163,017 or enalprilat. The depressor responses to bradykinin were similar before and after administration of vehicle or L-163,017. As expected, there was a significant increase in bradykinin-induced hypotension for all doses of bradykinin following enalaprilat (Table 2). The baseline renin-angiotensin system for the enalaprilat-treated animals was significantly less than that of the L-163,017 group both prior to and following treatment ($P < 0.05$).

4. Discussion

The data from the present series of experiments clearly demonstrate that L-163,017 is a potent, orally active, nonpeptide angiotensin II receptor antagonist with balanced affinity for both the angiotensin AT_1 and AT_2 receptors. The in vitro binding data, in rat adrenal, show that the ratio of the IC_{50} values at the angiotensin AT_1/AT_2 receptors is very close to unity (0.76) (Chang et al., 1995). Importantly, this ratio of angiotensin AT_1/AT_2 receptor binding is retained following administration to both rats and dogs. Because L-163,017 remains balanced in vivo, the inhibition of the pressor response to angiotensin II (angiotensin AT_1 receptor mediated) can be used as an indication of receptor occupancy at both the angiotensin AT_1 and AT_2 receptors and assumed blockade of angiotensin AT_2 receptors. Since there is currently no agreed-upon biological response to angiotensin AT_2 receptor stimulation, we cannot determine that L-163,017 is completely without 'agonist activity' at the angiotensin AT_2 receptor site. However, data from the present series of experiments demonstrate that L-163,017 is without agonist activity at the angiotensin AT_1 receptor. Because L-163,017 is an antagonist at the angiotensin AT_1 receptor, it is reasonable to assume it is also an antagonist at the angiotensin AT_2 receptor site until a reproducible biological response to angiotensin AT_2 receptor stimulation allows testing for agonist activity. Therefore, L-163,017 provides researchers with an important new tool for studying the role of the renin-angiotensin system as well as a potentially important new therapeutic agent for the treatment of disease.

Previous work from these laboratories has shown that incorporation of a 6-amido substituent into angiotensin AT_1 receptor-selective quinazolinone antagonists led to an extraordinary enhancement of angiotensin AT_2 receptor antagonist potency, resulting in the discovery of the first potent, balanced nonpeptidic antagonist, L-159,689 (De Laszlo et al., 1993). L-159,689 is orally active in rats, blocking pressor response to angiotensin II at 3 mg/kg (70% peak inhibition, duration > 6 h). However, this antagonist (although orally active) did not show acceptable duration of action in dogs and therefore was not suitable for further in vivo pharmacologic study.

Overlays of the chemical structures of L-159,689 and the angiotensin AT_1 receptor-selective antagonist MK-996 (Kivlighn et al., 1995) suggested that incorporation of a similar amido substituent on the imidazopyridine ring might likewise increase angiotensin AT_2 receptor binding potency. This expectation was borne out by the markedly increased angiotensin AT_2 receptor antagonist affinity found for 6-amido analogs of MK-996 (Mantlo et al., 1994). Enhancements in angiotensin AT_2 receptor antagonist affinity were also achieved by

modification of the acylsulfonamide moiety of MK-996 (Naylor et al., 1994). Particularly advantageous in this regard were sulfonylcarbamate groups (Glinka et al., 1994). Incorporation of both an amido substituent and a sulfonylcarbamate group led to a series of potent, balanced imidazopyridine antagonists from which L-163,017 was chosen for in-depth study.

The in vitro and in vivo potency of L-163,017 is greater than that of losartan. Compared with MK-996 the in vitro potency of L-163,017 is greater (angiotensin AT_1 receptor); however, the in vivo potency is less. Although the present study was not designed to address this issue, a possible explanation may be found in the effect of plasma protein on the binding affinity of the two compounds. In the presence of human serum albumin, the potency (IC_{50} at the angiotensin AT_1 receptor) of MK-996 is reduced from 0.36 to 25 nM (i.e. 69-fold) whereas L-163,017 is reduced from 0.13 to 52 nM (i.e. 408-fold). The importance of the effect of human serum albumin is not clear. It should also be noted that the in vitro potencies of losartan and E3174 are also reduced in the presence of human serum albumin (15 and 250 times, respectively); yet losartan has been shown to block the pressor response to angiotensin II and to be an effective antihypertensive agent in man (Nelson et al., 1992). Therefore, while we do not completely understand the role that human serum albumin may play in the biological effectiveness of these compounds, it appears that the absolute potency in the presence of human serum albumin is perhaps not as important as the relative change or shift from the potency in the absence of human serum albumin. Further, it appears that a shift in excess of 400-fold can result in a significant potency difference in vivo.

Many physiological roles of the angiotensin AT_2 receptor have been proposed and recently reviewed (Timmermans et al., 1993). In the present series of experiments, L-163,017 was found to be a potent antihypertensive agent in a renin-dependent model of hypertension. However, the antihypertensive efficacy was no greater than that of enalapril or MK-996 when administered orally at 3.0 mg/kg. These findings are in agreement with reports from other laboratories which have failed to demonstrate a significant difference between these classes of compounds when a reduction of blood pressure is used as the primary end point (Smith et al., 1992; Wood et al., 1990; Bunkenburg et al., 1991; Wong et al., 1990). Therefore, these data do not support an important role of the angiotensin AT_2 receptor in the acute control of blood pressure. However, it should be pointed out that potentially important renal effects have been attributed to blockade of the angiotensin AT_2 receptor (Keiser et al., 1992; Cogan et al., 1991). The importance of the kidney in the long-term regulation of arterial pressure is well established: therefore, chronic administration of a balanced angiotensin II receptor antagonist such as L-163,017 may reveal advantages over other therapies. However, a recent report has suggested that stimulation of the angiotensin AT_2 receptor may have an antiproliferative effect on vascular smooth muscle growth which would counteract the growth promoting action of angiotensin AT_1 receptor stimulation (Nakajima et al., 1994). Therefore, the results of experiments in which compounds like L-163,017 are administered over long periods of time will further our understanding of the role of the angiotensin AT_2 receptor in cardiovascular homeostasis as well as growth and development.

Although angiotensin converting enzyme inhibitors have demonstrated clinical efficacy in the treatment of hypertension and heart failure; they are not physiologically specific for the renin-angiotensin system. In addition to the blockade of the formation of angiotensin II from angiotensin I, angiotensin converting enzyme inhibitors also prevent the degradation of bradykinin (Carretero et al., 1981). The importance of bradykinin in the antihypertensive efficacy of angiotensin converting enzyme inhibitors has been the subject of much debate. Also, the potentiation of bradykinin has been suggested to be responsible for some of the side effects reported during angiotensin converting enzyme inhibitor therapy. The reported side effects include angioneurotic edema, dry cough, and urticarial rash (Robertson, 1987). These side effects have led to difficulties with patient compliance and in some cases, discontinuation of therapy. Because angiotensin II receptor antagonists bind directly to the angiotensin receptor, they are not expected to potentiate the effects of kinins. L-163,017 does not potentiate the hypotensive effects of bradykinin, strongly indicating that L-163,017 does not interfere with the breakdown and metabolism of kinins. The finding that the antihypertensive efficacy of L-163,017 is not different from that of an angiotensin converting enzyme inhibitor suggests that the potentiation of bradykinin by angiotensin converting enzyme inhibitors does not play an important role in their antihypertensive effect. Therefore, it is expected that L-163,017 will have similar antihypertensive efficacy to that of an angiotensin converting enzyme inhibitor and will be devoid of the previously mentioned side effects.

In summary, L-163,017 is a potent nonpeptide angiotensin II receptor antagonist with balanced affinity for both the angiotensin AT_1 and AT_2 receptors. L-163,017 is orally bioavailable and remains balanced following in vivo administration. L-163,017 does not potentiate the effects of bradykinin and, therefore, is not expected to produce the kinin-related side effects often observed with angiotensin converting enzyme inhibitors. The antihypertensive efficacy of L-163,017 following acute administration is not different from

that of an angiotensin converting enzyme inhibitor or an angiotensin AT_1 receptor-selective antagonist indicating that neither bradykinin nor the angiotensin AT_2 receptor plays an important role in the acute reduction in blood pressure produced by angiotensin converting enzyme inhibitors or angiotensin II receptor antagonists. However, angiotensin AT_2 receptor stimulation may have antiproliferative effects which may be beneficial in preventing the vascular remodeling associated with various cardiovascular diseases. Therefore, further studies directed at the effects of chronic administration of a balanced angiotensin II receptor antagonist, such as L-163,017, certainly appear warranted.

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