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L-163,491 is a partial angiotensin AT_1 receptor agonist in the hindquarters vascular bed of the cat

Bracken J. De Witt, Etoi A. Garrison, Hunter C. Champion, Philip J. Kadowitz *

Department of Pharmacology SL83, Tulane University School of Medicine, 1430 Tulane Avenue, New Orleans, LA 70112, USA

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Abstract

Responses to the nonpeptide angiotensin II agonist 5,7-Dimethyl-2-ethyl-3-[[2′-([butyloxycarbonyl) aminosulfonyl]-5′-(3-methyoxybenzyl)-[1,1′-biphenyl]-4-yl]methyl]-^{3}H-imidazo[4,5-b]pyridine (L-163,491) were investigated and compared with responses to angiotensin II, angiotensin IV and norepinephrine in the hindquarters vascular bed of the cat under constant-flow conditions. Injections of L-163,491 into the hindquarter perfusion circuit caused dose-related increases in hindquarters perfusion pressure. In relative terms, angiotensin II was more potent than norepinephrine, which was more potent than angiotensin IV and L-163,491 in increasing hindlimb vascular resistance. The slope of the dose–response curve for L-163,491 was flat, while the apparent affinity of the compound for angiotensin AT_1 receptors was slightly greater than angiotensin IV. Responses to L-163,491 were inhibited by the angiotensin AT_1 receptor antagonist DuP 532 (2-propyl-4-pentafluoroethyl-1-[2′-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole-5-carboxylic acid) and were not altered by the angiotensin AT_2 receptor antagonist PD123,319 (S(+)-1-[[4-(Dimethylamino)-3-methylphenyl]methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-6-carboxylic acid ditribluoroacetate). However, the increase in hindlimb perfusion pressure in response to angiotensin II and angiotensin IV was significantly decreased following injection of L-163,491. These data suggest that the nonpeptide angiotensin analog L-163,491 has partial agonist activity, which is dependent on the stimulation of angiotensin AT_1 receptors in the hindquarters vascular bed of the cat.

Keywords: Angiotensin; Nonpeptide angiotensin receptor agonist; (Feline); DuP 532; PD123,319

1. Introduction

Angiotensin II is the principal biologically active peptide of the renin–angiotensin system (Cheng et al., 1994). This octapeptide plays an important role in the regulation of vasomotor tone, body fluid balance, and electrolyte homeostasis (Regoli et al., 1974; Peach, 1977). Several classes of pharmacologic agents, including angiotensin-converting enzyme inhibitors and angiotensin receptor antagonists, which alter the activity of the renin–angiotensin system, are used in the treatment of hypertension and congestive heart failure (Osei et al., 1993; Awan and Mason, 1996; De Witt et al., 1996; McConnaughey et al., 1999).

The development of potent nonpeptide angiotensin II receptor antagonists has led to the identification of at least two angiotensin II receptor subtypes, angiotensin$_1$ (AT_1) and angiotensin$_2$ (AT_2) receptors (Bottari et al., 1993). Angiotensin AT_1 receptors have been isolated from many tissues, including vascular smooth muscle, while angiotensin AT_2 receptor transcripts have been described in bovine cerebellum and uterus, as well as in rat adrenal medulla (Zhuo et al., 1998). The function of the angiotensin AT_2 receptor in vivo is uncertain, however, it has been reported that angiotensin AT_2 receptors play a role in fetal development and may mediate vasodepressor responses to angiotensin II in the systemic vascular bed of the rat, although this has been disputed recently (Timmermans et al., 1993; Champion et al., 1998). The angiotensin AT_1 receptor is believed to be responsible for most, if not all, of the cardiovascular and renal responses to angiotensin II (Timmermans et al., 1993).

In addition to the development of nonpeptide angiotensin receptor antagonists, a new class of nonpep

* Corresponding author. Tel.: +1-504-588-5444; fax: +1-504-588-5283.

E-mail address: pkadowi@tulane.edu (P.J. Kadowitz).

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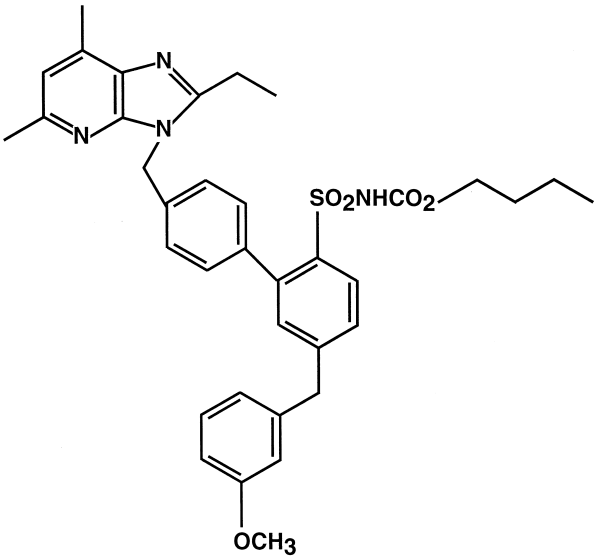

Fig. 1. Chemical structure of L-163,491.

tide receptor agonists has been developed. 5,7-Dimethyl-2-ethyl-3-[[2′-([butyloxycarbonyl) aminosulfonyl]-5′-(3-methyoxybenzyl)-[1,1′-biphenyl]-4-yl]methyl]-^{3}H-imidazo-[4,5-b]pyridine (L-163,491) is a nonpeptide angiotensin receptor agonist. L-163,491 has been shown to increase systemic pressure in the rat and pulmonary arterial pressure in the cat by an angiotensin AT_1 receptor mechanism (Huckle et al., 1994; Kaye et al., 1995a,b). However, L-163,491 has also been shown to competitively antagonize angiotensin II-induced increases in inositol phosphate production (Huckle et al., 1994). Furthermore, biphenylimidazole compounds have been demonstrated to have dual agonistic and antagonistic properties (Perlman et al., 1997).

While the effects of L-163,491 have been studied in the systemic vascular bed of the rat and the pulmonary vascular bed of the cat, little, if anything, is known about the effects of L-163,491 on perfusion pressure in the feline hindlimb vascular bed and if this compound has antagonistic activity (Huckle et al., 1994; Kaye et al., 1995a,b). Therefore, the present study was undertaken to investigate the effects of L-163,491 on responses to angiotensin II and angiotensin IV in the hindlimb vascular bed of the cat.

2. Methods

Adult cats, unselected as to sex weighing 2.5–4.0 kg, were sedated with ketamine hydrochloride (10–15 mg/kg i.m.) and were anesthetized with pentobarbital sodium (30 mg/kg i.v.). Additional doses of pentobarbital were given as needed to maintain a uniform level of anesthesia. The trachea was cannulated, and the animals were ventilated with a Harvard model 607 ventilator at a volume of 40–60 ml at 15–22 breaths/min. Catheters were placed into an external jugular vein for the i.v. administration of drugs and into a carotid artery for the measurement of systemic (aortic) arterial pressure. For constant-flow perfusion of the hindquarters (hindlimb) vascular bed, a 3–4 cm segment of the distal abdominal aorta was exposed through a ventral midline incision and cleared of surrounding connective tissue. All branches of the aorta distal to the origin of the external iliac arteries were ligated to restrict blood flow to the hindlimbs. Following administration of heparin sodium (1500 U/kg i.v.), the aorta was ligated and catheters were inserted into the aorta proximal and distal to the ligature. Blood was withdrawn from the proximal catheter and pumped at a constant rate with a Sigmamotor model T-8 pump into the distal aortic catheter. Perfusion pressure was monitored from a lateral tap in the perfusion circuit between the pump and the distal catheter. Hindlimb perfusion pressure and systemic arterial pressure were measured with Statham P23 transducers and were recorded on a Grass model 7 polygraph. The flow rate was set so that hindlimb perfusion pressure approximated systemic arterial pressure and was not changed throughout the course of the experiment. The flow rate was determined by timed collection and ranged from 24 to 32 ml/min. The hindlimb vascular bed was denervated by ligating and cutting the lumbar sympathetic chain ganglia between L3 and L4. Female animals were determined to be nonpregnant by abdominal exploration. These procedures have been described previously (De Witt et al., 1996).

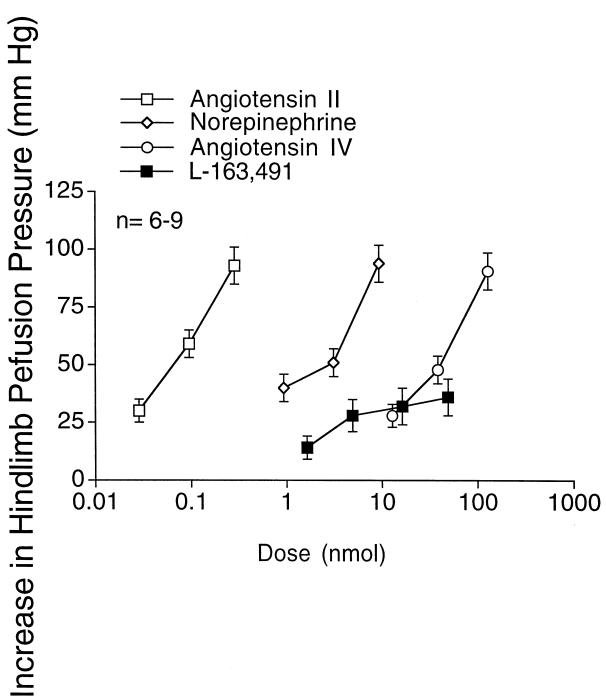

Fig. 2. Dose–response curves comparing increases in hindlimb perfusion pressure in response to angiotensin II, norepinephrine, angiotensin IV and L-163,491. The compounds were injected into the perfusion circuit in small volumes in a randomized sequence. Doses of compounds are expressed on a molar basis. *n* indicates the number of experiments.

In this study, three series of experiments were carried out. In the first series of experiments, the effects of L-163,491 in doses of 3–300 μg i.v. on hindlimb perfusion pressure were investigated. Vasoconstrictor responses to L-163,491 were compared with responses to angiotensin II, angiotensin IV and norepinephrine when the agonists were administered on a nanomole basis to take molecular weight into account. In a separate set of experiments, the influence of the angiotensin AT_1 receptor antagonist DuP 532 (2-propyl-4-pentafluoroethyl-1-[2′-(1H-tetrazol-5-yl)-biphenyl-4-yl)methyl]imidazole-5-carboxylic acid) and the angiotensin AT_2 receptor antagonist PD123,319 (S(+)-1-[[4-(Dimethylamino)-3-methylphenyl]methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazo[4,5-c] pyridine-6-carboxylic acid ditribluoroacetate) on vasoconstrictor responses to L-163,491 were investigated in the hindlimb vascular bed of the cat. In the third series of experiments, the influence of L-163,491 (40 μg/kg i.v.) and angiotensin IV (40 μg/kg i.v.) injections on hindlimb vasoconstrictor responses to angiotensin II, angiotensin IV and norepinephrine was investigated in the hindlimb vascular bed.

The agonists were injected directly into the hindquarters perfusion circuit distal to the pump in a random sequence in small volumes (30–100 μl). The antagonists used in these experiments were injected intravenously. Angiotensin II, angiotensin IV and norepinephrine (Sigma, St. Louis, MO), DuP 532 (Dupont Merck, Wilmington, DE) and PD123,319 (Parke-Davis, Ann Arbor, MI) were dissolved in 0.9% NaCl. L-163,491 (Merck Research Laboratories, Rahway, NJ) was prepared in 0.1 ml dimethylsulfoxide and 0.9 ml of 100% ethanol at a concentration of 10 mg/ml, and dilutions were made with normal saline. Working solutions of all agonists were prepared on a frequent basis, stored in brown stoppered bottles, and kept on crushed ice during an experiment. The hemodynamic data are expressed as mean ± S.E. and were analyzed using a one-way analysis of variance and Scheffe′s *F*-test or a paired *t*-test (Snedecor and Cochran, 1980). A *P* value of less than 0.05 was used as the criterion for statistical significance.

3. Results

The chemical structure of L-163,491 is shown in Fig. 1, and responses to the nonpeptide angiotensin receptor agonist were investigated in the hindquarters vascular bed of

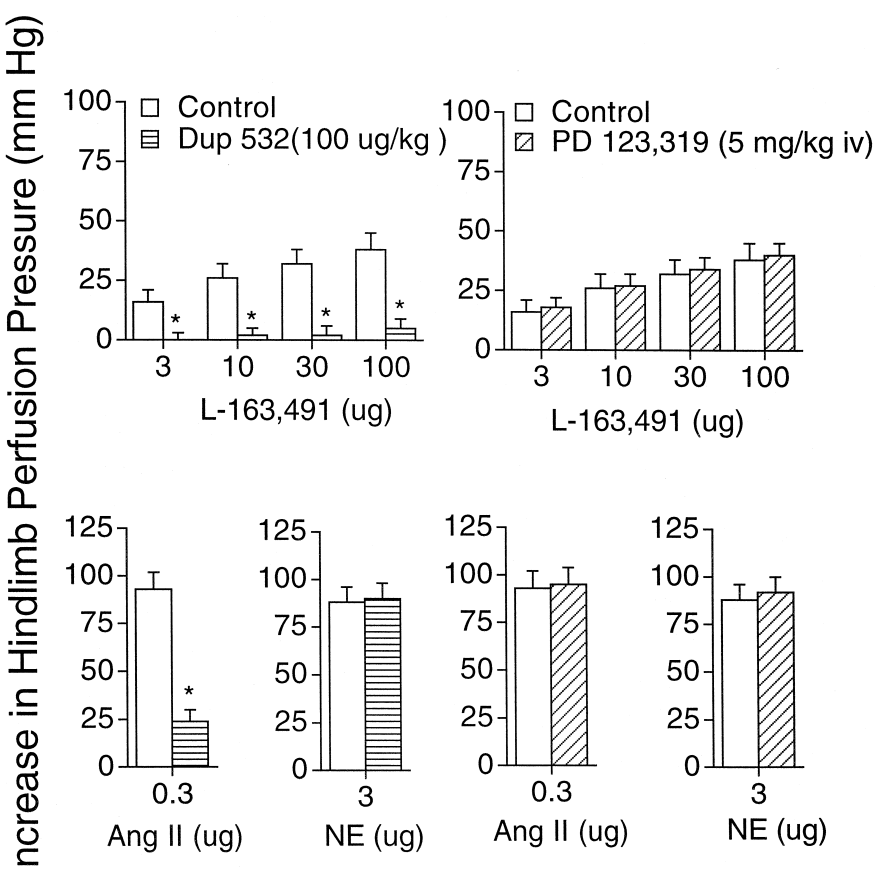

Fig. 3. Influence of DuP 532 (A) and PD 123,319 (B) on vasoconstrictor responses to L-163,491, angiotensin II and norepinephrine in the hindquarters vascular bed. Compounds were injected directly into the perfusion circuit and responses were determined before and beginning 10–15 min after administration of DuP 532 and PD123,319 in doses of 100 μg/kg i.v. and 5 mg/kg i.v., respectively. Number of experiments equals six. * Significantly different from control.

the cat. Under conditions of controlled blood flow, injections of L-163,491 in doses of 3–100 μg into the hindlimb perfusion circuit significantly increased hindlimb perfusion pressure and these data are summarized in Fig. 2. When doses of L-163,491, angiotensin II, angiotensin IV and norepinephrine are expressed on a nanomole basis, L-163,491 was found to be significantly less potent than angiotensin II and norepinephrine in increasing hindlimb perfusion pressure. The dose–response curve for L-163,491 was not parallel to the dose–response curves for angiotensin II and angiotensin IV. The slope of the dose–response curve for L-163,491 was flatter and the doses of the nonpeptide angiotensin analog required to increase perfusion pressure were two to three orders of magnitude higher than the doses of angiotensin II. The approximate peak increase in perfusion pressure induced by L-163,491 was 60% less than that produced by angiotensin II (0.3 μg). Angiotensin IV in doses of 10–100 μg caused dose-related increases in perfusion pressure, and the slope of the curves for angiotensin II and angiotensin IV were similar with angiotensin II being two to three orders of magnitude more potent (Fig. 2). Norepinephrine in doses of 1–10 μg caused dose-related increases in perfusion pressure and was more potent than angiotensin IV but less potent than angiotensin II (Fig. 2). The apparent affinity of angiotensin II was approximately twofold greater than L-163,491, which was slightly greater than angiotensin IV.

The angiotensin II receptor subtype mediating vasoconstrictor responses to L-163,491 was investigated, and these data are summarized in Fig. 3. Following administration of

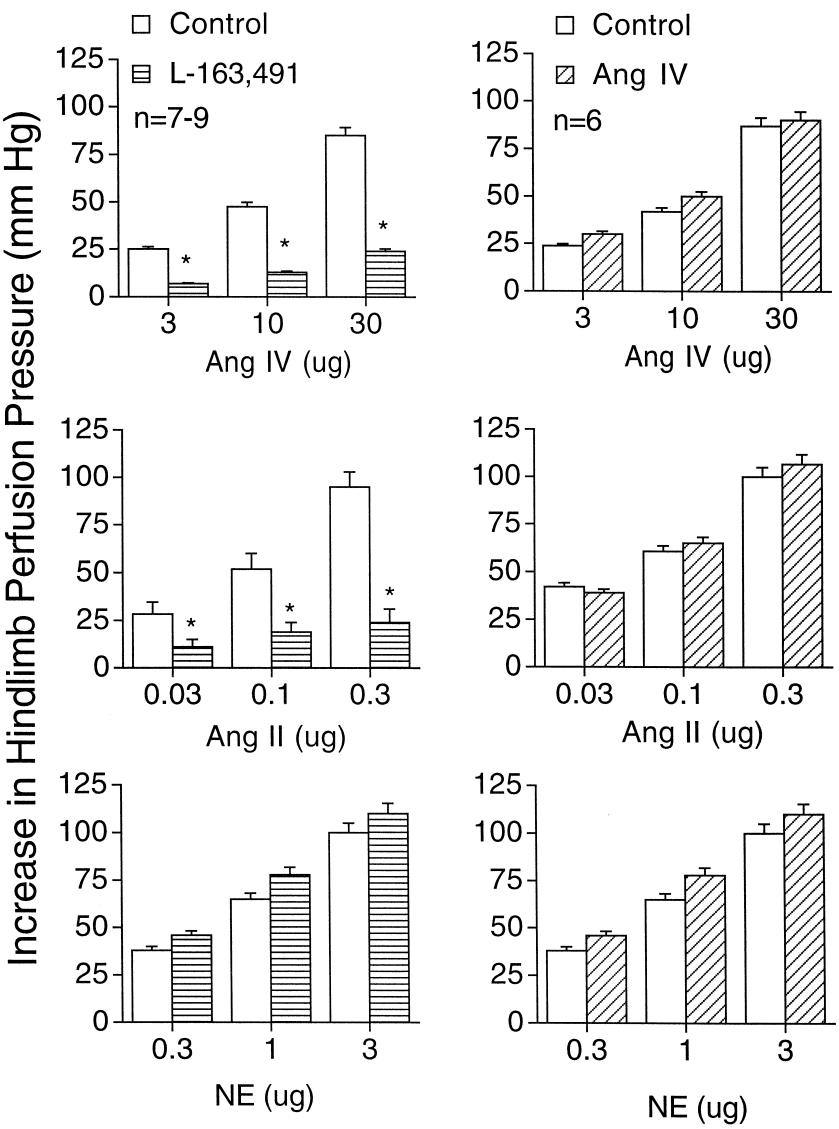

Fig. 4. Influence of L-163,491 (A) and angiotensin IV (B) on vasoconstrictor responses to angiotensin II (Ang II), angiotensin IV (Ang IV), and norepinephrine (NE) in the hindquarters vascular bed. Compounds were injected directly into the perfusion circuit and responses were determined before and beginning 5–10 min after administration of L-163,491 and angiotensin IV in a dose of 40 μg/kg i.v. *n* indicates the number of experiments. * Significantly different from control.

the angiotensin AT_1 receptor antagonist DuP 532 in a dose of 100 μg/kg i.v., increases in hindlimb perfusion pressure in response to L-163,491 were significantly reduced (Fig. 3). Following administration of the angiotensin AT_2 receptor antagonist PD123,319 in a dose of 5 mg/kg, increases in hindlimb perfusion pressure in response to L-163,491, angiotensin II, angiotensin IV and to norepinephrine were not altered (Fig. 3).

The observation that the slope of the dose–response curve for L-163,491 was flat and that the apparent affinity of the compound was slightly greater than for angiotensin IV suggests that this analog may be a partial agonist and, thus, may also act as an antagonist for peptides with higher efficacy. Therefore, the effect of L-163,491 on responses to angiotensin II was investigated, and these data are summarized in Fig. 4. Responses to angiotensin II were compared before and during administration of L-163,491 (40 μg/kg i.v.). The administration of L-163,491 had no significant effect on baseline hindlimb perfusion pressure. However, during the treatment period, vasoconstrictor responses to angiotensin II and angiotensin IV were reduced significantly, whereas responses to norepinephrine were not changed (Fig. 4). Following administration of L-163,491, responses to higher doses of angiotensin II returned toward control values such that responses to the 1 and 3 μg doses were approximately equal to responses at the 0.03 and 0.1 μg doses, respectively (data not shown).

In order to determine if angiotensin IV also has antagonistic activity similar to L-163,491, the effects of angiotensin IV (40 μg/kg i.v.) on increases in hindlimb perfusion pressure in response to angiotensin II, angiotensin IV and norepinephrine were investigated. These results are summarized in Fig. 4, and following administration of angiotensin IV into the perfusion circuit, vasoconstrictor responses to angiotensin II, angiotensin IV, and norepinephrine were not altered (Fig. 4).

4. Discussion

Results of the present study show that the nonpeptide angiotensin II receptor agonist L-163,491, angiotensin II, angiotensin IV and norepinephrine produce dose-related increases in hindquarters perfusion pressure in the cat. Inasmuch as blood flow was maintained constant, the increases in perfusion pressure reflect increases in hindquarters vascular resistance. Vasoconstrictor responses to L-163,491 were inhibited by the angiotensin AT_1 receptor antagonist DuP 532 in a dose that antagonized responses to angiotensin II and angiotensin IV, but not to norepinephrine. Hindquarters vasoconstrictor responses to L-163,491 were not altered by the angiotensin AT_2 receptor antagonist PD123,319. These results suggest that vasoconstrictor responses to L-163,491 are mediated by the activation of angiotensin AT_1 receptors. These results support studies in the rat and pulmonary vascular bed of the cat in which pressor responses to L-163,491 were significantly reduced by angiotensin AT_1 receptor antagonists (Huckle et al., 1994; Kaye et al., 1995a,b).

L-163,491 is a nonpeptide angiotensin II receptor agonist, which in membrane preparations, has been reported to be selective for the angiotensin AT_1 receptor (Kivligh et al., 1995; Perlman et al., 1995). In terms of relative pressor activity in the hindlimb vascular bed, L-163,491 (100 μg) was observed to be more than 100-fold less potent than angiotensin II. The nonpeptide angiotensin II analog produced an increase in hindlimb perfusion pressure, which was approximately 60% less than that of angiotensin II (0.3 μg) and angiotensin IV (30 μg).

It has also been reported that dose–response curves for certain nonpeptide biphenylimidazole compounds are bell-shaped in vitro (Perlman et al., 1997). There are several possible explanations for a bell-shaped dose–response curve (Ariëns 1954; Szabadi, 1977; Rovati and Nicosia, 1994). However, in the present study, the dose–response curve for L-163,491 was flat in nature. The flatness of the dose–response curve, while maintaining affinity for the angiotensin AT_1 receptor, suggests that L-163,491 may act as a partial agonist in the hindlimb vascular bed of the cat. The reason for the difference between the dose–response curve for L-l63,491 in vivo and the dose–response curve in the in vitro setting is unknown. However, it is probably related to low basal stimulation of the angiotensin AT_1 receptor in maintaining baseline hindlimb perfusion pressure. This low level of AT_1 stimulation makes it difficult to observe the down-sloping portion of the dose–response curve. This is supported by the observation that nonpeptide angiotensin II receptor antagonists, such as DuP 532, do not significantly decrease baseline pressure in the hindlimb vascular bed of the cat (Osei et al., 1993).

The present study suggests that L-163,491 may be a partial agonist in the regional vascular bed of the cat. Stephenson (1956) is credited with modifying Clark's receptor theory to include a broader definition of the terms efficacy and affinity and the discussion of the term "partial agonist". Stephenson proposed three central hypotheses: (1) an agonist may produce a maximal effect by occupying only a small portion of the receptors; (2) the response to an agonist may not be linearly proportional to the number of receptors occupied; and (3) drugs are variable in their ability to initiate a biological response and, as a result, may bind different numbers of receptors to produce responses of equal magnitude. Partial agonists may be defined as agonists that produce a submaximal biological response at full receptor occupancy and competitively block the effect of agonists of higher intrinsic activity (Kenakin, 1993; Bourne and Roberts, 1995). As a result, depending on the dose and the number of receptors occupied, a partial agonist may antagonize pressor responses to a full agonist. The explanation for L-163,491 acting as a partial agonist is unknown but could relate to its chemical nature in relation to the angiotensin AT_1 receptor. Biphenylimidazole compounds were developed from align-

ing their imidazole structure with the imidazole of His^6 in angiotensin II (Nirula et al., 1996). It has been suggested that certain biphenylimidazole compounds do not interact with the Asp^{281} side chain of the angiotensin AT_1 receptor, thereby decreasing their potency (Feng et al., 1995). Furthermore, the binding requirements for L-163,491 to the angiotensin AT_1 receptor are not the same as for other biphenylimidazole compounds, suggesting that its chemical nature is different from other substances in its chemical class (Hunyady et al., 1998).

In the present study, L-163,491 significantly reduced pressor responses to angiotensin II and angiotensin IV, while pressor responses to norepinephrine were unaltered. This antagonistic activity was selective in that administration of angiotensin IV in a similar dose did not inhibit pressor responses to angiotensin II, angiotensin IV, and norepinephrine in the hindlimb vascular bed. These data extend previous findings in which L-163,491 was found to competitively antagonize angiotensin II-induced increases in inositol phosphate production (Huckle et al., 1994).

It has been previously demonstrated in vitro that certain biphenylimidazole compounds have dual agonistic and antagonistic properties at the angiotensin AT_1 receptor (Perlman et al., 1997). This study suggested that a single methyl group difference between biphenylimidazole compounds determined whether these substances act as antagonist or agonist. Furthermore, the difference in the methyl group did not alter the affinity of the compounds for the angiotensin AT_1 receptor. In the present study L-163,491 acted as a partial agonist (having both properties of an agonist and antagonist), depending on the state of angiotensin AT_1 receptor stimulation in the hindlimb vascular bed.

Certain biphenylimidazole compounds have been reported to be insurmountable antagonists in vitro (Perlman et al., 1997). However, in the present study, following administration of larger doses of L-163,491, the vasoconstrictor response to angiotensin II returned toward control value, suggesting that inhibition of angiotensin II-mediated responses is competitive in nature. The reason for the difference in the results in this study and the previous study is unknown, but may be due to differences in chemical structure of the biphenylimidazole compounds, differences in degradation of the biphenylimidazole compounds, or differences in the nature of the angiotensin AT_1 receptors studied.

In conclusion, the results of the present investigation demonstrate that L-163,491 produces dose-related increases in hindquarters perfusion pressure in the cat. Angiotensin II was more potent than norepinephrine, which was more potent than angiotensin IV and L-163,491. The slope of dose–response curve for L-163,491 was flat, while the apparent affinity of the compound for the angiotensin AT_1 receptor was slightly greater than angiotensin IV. The vasoconstrictor response to L-163,491 was selectively inhibited by angiotensin AT_1 receptor antagonists, but was not altered by blockade of the angiotensin AT_2 receptor. The increase in hindlimb perfusion pressure in response to angiotensin II and angiotensin IV was significantly decreased following injection of L-163,491. These data suggest that the nonpeptide angiotensin analog L-163,491 has partial agonist activity, which is dependent upon stimulation of angiotensin AT_1 receptors in the hindquarters vascular bed of the cat.

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